Remarks/Arguments:

Claim 12, currently amended, claims 13-20, previously presented, and new claim 21 are pending.

Claims 1-11 are canceled, without prejudice or disclaimer.

Claim 12 is amended—as are claims 13-20, being dependent on claim 12—by limiting the claimed process to treatment of a "non-human animal," i.e. the claimed subject matter no longer literally covers treatment of a human.

New (independent) claim 21 provides:

Process for treating lameness caused by osteoarthrosis comprising the administration to a horse, not suffering from fractures, of an effective amount of 4-chlorophenylthiomethylenebisphosphonic acid or its sodium salt.

In other words, present claim 21 is limited to treatment of a specific animal, i.e., "a horse," by administering—to the horse—the specific compound "4-chlorophenylthiomethylenebisphosphonic acid" or the "sodium salt" of the compound (see, e.g., original claims 6 and 10).

Claims 12-20 were under final rejection based on 35 USC 103(a) as being allegedly unpatentable over US 4473560 (Biere) in view of US 5488041 (Barbier) and further in view of The American Journal of Medicine, 101, 1996, 339-340 (Siris). Reconsideration is requested, in view of the changes to the claims, effected hereby, and the following remarks.

An obviousness, i.e., § 103(a), analysis requires determining every difference between the claims and the cited prior art. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 230 USPQ 416 (Fed. Cir. 1986). To establish *prima facie* obviousness of a claimed invention, all the claim

limitations must be taught or suggested by the prior art. *In re Royka*, 180 USPQ 580 (CCPA 1974). A "ground of rejection is simply inadequate on its face . . . [when] the cited references do not support each limitation of [the] claim." *In re Thrift*, 63 USPQ2d 2002, 2008 (Fed. Cir. 2002).

As indicated, above, the present claims are limited solely to the treatment of a "non-human animal." None of the cited references—taken together or separately—teaches or suggests the limitation "administration, to a non-human animal"—of the recited "bisphosphonic acid derivative" for the recited "treating lameness caused by osteoarthrosis—in accordance with the present claims.

Siris, first of all, is limited exclusively to the treatment of humans. That is, Siris discloses the treatment of Paget's disease—an exclusively human affliction, having no equivalent in veterinary medicine—by administering a single bisphosphonic acid derivative, i.e., alendronate. That Paget's disease afflicts only humans is inferentially evidenced by the reference, itself; Siris never even mentions anything but the treatment of humans. And, in fact, nothing of record teaches or suggests—even remotely—that any animals other than humans can have Paget's disease

In the context of a rejection for obviousness under §103, the "Examiner bears [both] the initial burden . . . of presenting a *prima facie* case of unpatentability" and "the ultimate burden of persuasion on the issue." *In re Oetiker*, 24 USPQ 1443, 1444 and 1447 (Fed. Cir. 1992). "The Examiner can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant references." *Ex parte Obukowicz*, 27 USPQ2d 1063, 1065 (BPA&I 1992) *(emphasis, added)*. The "evidence upon which the examiner relies must clearly indicate that a

worker of routine skill in this art would view the claimed invention as being obvious." *Ex parte*

Wolters, 214 USPQ 735, 736 (BPA&I 1982).

"It is facts which must support the legal conclusion of obviousness." *Ex parte Crissy*, 201

USPQ 689, 695 (POBdApp 1976).

> The Patent Office has the initial duty of supplying the factual basis for its rejection.
> It may not, because *it* may *doubt* that the invention is patentable, resort to
> speculation, unfounded assumptions or hindsight reconstruction to supply
> deficiencies in the factual basis.

In re Warner, 154 USPQ 173, 178 (CCPA 1967) (*emphasis in original*). An argument by the PTO

"is not prior art." *In re Rijckaert*, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993). When the

> USPTO asserts that there is an explicit or implicit teaching or suggestion in the prior
> art, it must indicate where such a teaching or suggestion appears in the reference.

28 USPQ2d at 1957. To support a rejection for obviousness based on the combination of separate

prior art teachings, the PTO "must identify specifically the principle, known to one of ordinary skill,

that suggests the claimed combination." *In re Rouffet*, 47 USPQ2d 1453, 1459 (Fed. Cir. 1998)

(emphasis added).

Although the PTO has not satisfied its initial burden, *i.a.*, of demonstrating that Paget's

disease afflicts non-human animals—and, so, has failed to show how the skilled artisan would have

combined Siris with teachings in the other cited references concerning the treatment of non-human

animals—applicants provide, herewith, the Rule 132 declaration of Dr. Dominique Thibaud and

copies of:

    — Selby, *Bone*, vol. 31, n°, 2002, 10-19;

- Hamdy, *Biosphosphonates on Bones*, chap. 17, Paget's disease of bone:

  Symptoms, Signs and Morbidity, 1995, Elsevier Sciences B.V.;

- Resnick: Paget's disease of bone: Current Status and a look back to 1943 and

  earlier, *AJR*, 150, 249-256, 1988; and

- Frame et al., Paget's disease: a review of current knowledge, *Diagnostic*

  Radiology, 141: 21-24, 1981.

As stated in the Rule 132 declaration (*emphasis in original*):

Siris teaches only the treatment of human patients for Paget's disease since—as
would be understood by the skilled artisan—only humans *can be treated* for Paget's
disease. Paget's disease is a purely human affliction, having no equivalent in
veterinary medicine. There is nothing cited in the prosecution history—nor could
there be—showing that Paget's disease is an affliction of non-human animals.
Therefore, the teachings of Siris are strictly limited to the treatment of human
patients.

Per amendment to be filed concurrently herewith, the rejected claims are amended
as in the attached Appendix. As indicated in the Appendix, claims 12-20, as
amended, are limited to treatment of a <u>non-human</u> animal; thereby, rendering
irrelevant—to amended claims 12-20—the teachings Siris (limited to humans), as
relied on in the rejection.

Accordingly, the Rule 132 declaration evidences that Paget's disease is an exclusively human

affliction and, therefore, the teachings of Siris—limited strictly to the treatment of Paget's

disease—are irrelevant, i.e., Siris is not in the same filed of endeavor as the presently claimed

invention and, as such, cannot be combined with Biere and Brelier to establish obviousness of the

present claims under §103(a). *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 230 USPQ

416 (Fed. Cir. 1986).

The above listed references are all general reviews of Paget's disease. None of them mentions the occurrence of Paget's disease in animals. They all clearly teach that Paget's disease is a human affliction, the etiology of which is not elucidated.

The epidemiologic discussions in these references teach that Paget's disease affects elderly people, affects more men than women, has a genetic component, and is more prevalent in certain geographic areas. One could reasonably assume that, if Paget's disease afflicted animals as well as humans, these reviews would have at least mentioned it. However, none of these reviews of Paget's disease mentions anything about the disease in (non-human) animals and, as such, the reviews evidence that Paget's disease is solely a human disease.

This is confirmed in the study by Leach et al., "The genetics of Paget's disease of the bone," *The Journal of Clinical Endocrinology and Metabolism*, 86, 1, 24-28, 2001 (provided herewith). As the title suggests, the authors investigated the genetics of Paget's disease (PDB). They teach that Paget's disease has a familial tendency suggesting a genetic predisposition. Leach et al. (page 24, last paragraph of left column) state (emphasis added):

> The role of the major HLA [histocompatibility leucocyte antigen] loci on chromosome 6 in many human diseases makes it a logical starting point for studying the genetics of PDB.

Thus, the authors identify Paget's disease as one among "many human diseases" (emphasis added). Indeed, were Paget's disease an animal—as called as human—disease, the authors would have presumably investigated other targets, and/or pathways.

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Moreover Leach et al. (page 27, 1ˢᵗ column, lines 3-5 from the bottom) points out that there

is no existing <u>animal</u> model for Paget's disease, i.e.:

> However, until a virus is isolated or a paramyxoviral gene can be shown to induce
> PDB in an animal model, the role of the virus in the pathogenesis of PDB is still
> unclear.

There being no animal <u>model</u> of Paget's disease evidences that there is no Paget's disease in an

<u>animal</u>. Lack of animal model for Paget's disease is confirmed by Singer et al., "Paget's disease

of bone," in <u>Principles of Bone Biology</u>, Academic press, 1996, ch. 69, 969-977 (provided

herewith). Singer et al (971 of right column and 972 beginning of left column) to reproduce Paget's

disease in mice, following injections of pagetic bone extracts.

Singer et al. (971-973) also report a theory that infection of osteoclasts precursors by an

animal paramyxovirus, such as canine distemper virus, may lead in humans to Paget's disease, under

certain circumstances, such as genetic predisposition to the disease. Canine distemper is a highly

contagious virus disease, affecting dogs in particular, that attacks the respiratory, gastrointestinal,

integumentary, and CNS systems. Deem et al., *Journal of Zoo and Wildlife Medicine*, 31(4), 441-

451, 2000 (provided herewith). Canine distemper may also induce lesions of the bone metaphyses,

but their histological nature is different from the bone lesions observed in Paget's disease. According

to Singer et al., (973, beginning of right column): "The [canine distemper] histology does not

resemble Paget's disease." Therefore, there is no similarity between the clinical signs or bone

histological lesions associated with canine distemper infection in dogs and the ones in Paget's disease

in man.

Thus Siris—limited strictly to treating a human—is not relevant to the instant claims, which are limited to the treatment of a non-human animal.

Additionally, the "Response to Arguments" (final action, pages 2-3) contains an erroneous statement of law, erroneously characterizes applicants' arguments, and erroneously relies on a cited, court decision.

"An accidental or unwitting duplication of an invention" cannot defeat patentability. *In re Marshall*, 198 USPQ 344, 346 (CCPA 1978) (had anyone followed the teachings of the cited reference and received the benefit of the rejected claims, "it was an unrecognized accident," 198 USPQ at 346, because nothing in the reference inherently suggests such a benefit).

As stated in the Rule 132 declaration (emphasis in original):

> Siris teaches treating Paget's disease at an early stage, i.e., for the purpose of avoiding complications, such as osteoarthritis. Accordingly, patients treated according to Siris do not suffer from osteoarthritis, or from osteoarthritis-induced lameness, i.e., compounds are administered to prevent osteoarthritis—caused by Paget's disease—not to treat osteoarthritis. As a result, Siris neither teaches nor suggests the treatment of lameness caused by osteoarthrosis, as recited in the rejected (and amended) claims..

In the present case, the beneficial *use*—"treating lameness caused by osteoarthrosis"—recited in the instant claims is neither taught nor suggested by Siris. If anyone followed the teachings of the cited reference and received the beneficial use/effect set forth in the instant claims "it was an unrecognized accident," *Marshall*, 198 USPQ at 346. Since nothing in the reference inherently suggests the benefit "treating lameness caused by osteoarthrosis," as recited in the present claims, had the benefit occurred by following Siris it would have constituted "accidental or unwitting

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duplication" of the presently claimed invention and, therefore, cannot defeat patentability of the

present claims. *Marshall*, 198 USPQ at 346.

The erroneous statement of law evidences a misunderstanding, in general, of using the

doctrine of inherency to defeat patentability.

> The mere fact that a certain thing may result from a given set of circumstances is not
> sufficient to establish inherency.

Rijckaert, 28 USPQ2d at 1957(emphasis added). An argument by the PTO "is not prior art." 28

USPQ2d at 1957.

> That which may be inherent is not necessarily known. Obviousness cannot be
> predicated on what is unknown.

In re Spoormann, 150 USPQ 449, 452 (CCPA 1966).

> [A] retrospective view of inherency is not a substitute for some teaching or
> suggestion which supports the selection and use of the various elements in the
> particular claimed combination.

In re Newell, 13 USPQ2d 1248, 1250 (Fed. Cir. 1989).

In the present case, the benefit allegedly inherent in Siris was "not. . . known" and, so, the

alleged obviousness "cannot be predicated" on the teachings of Siris. *Spoormann*, 150 USPQ at 452.

Reliance on an unrecognized benefit—whether inherent or not—of Siris is a "retrospective view of

inherency," which "is not a substitute for some teaching or suggestion" in Siris "which supports" the

rejection. *Newell*, 13 USPQ2d at 1250. The inherency argument by the PTO "is not prior art."

Rijckaert, 28 USPQ2d at 1957. "New uses of old products or processes are indeed patentable subject

matter." *Perricone v. Medicis Pharmaceutical Corp.*, 77 USPQ2d 1321, 1328 (Fed. Cir. 2005).

PTO reliance on the *Keller* and *Merck & Co.* decisions—by the Federal Circuit and its predecessor Court—is misplaced. Applicants do not traverse the rejection by arguing simply that the rejected claims are distinguishable over each of the cited references taken individually. What applicants do argue is that there are <u>defects</u> in the individual references, which defects render the combination of these teachings—alleged by the PTO—incorrect. When a reference is relied on to meet limitations on a claim, in a rejection under §103(a) based on combined prior art teachings, "individual defects of the reference . . . can defeat the rejection." *In re Lyons*, 150 USPQ 741, 746 (CCPA 1966). *Ryko Manufacturing Co. v. NuStar, Inc.*, 21 USPQ2d 1053 (Fed. Cir. 1991).

For the foregoing reasons the rejection under §103(a) cannot be applied against any of the present claims. Withdrawal of the rejection appears to be in order.

Applicants also wish to point out that careful analysis of each of the three cited references shows how the statement of rejection does not correctly characterize each reference's teachings, i.e., as a whole. Teachings of the prior art must be <u>taken as a whole</u> in an obviousness analysis. *Ryko Manufacturing Co. v. Nu-Star, Inc.*, 21 USPQ2d 1053 (Fed. Cir. 1991).

> It is impermissible within the framework of §103 to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art.

In re Hedges, 228 USPQ 685, 687 (Fed. Cir. 1986). "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988).

Biere primarily concerns anti-inflammatory properties of bisphosphonic compounds of its formula (I). As correctly noted by the PTO, Biere does not teach or suggest the "bisphosphonic acid derivative"—defined by the recited Markush group of specific compounds—recited in the present claims.

Moreover, Biere merely teaches that the disclosed bisphosphonic compounds have inflammatory activity, rendering them useful for treating <u>inflammatory</u> arthritis. Incidentally, as previously shown (on the record), the term "arthritis"—by itself—is the commonly used term for <u>inflammatory</u> diseases such as <u>rheumatoid arthritis</u>; the term "arthritis" does not cover <u>non-</u>inflammatory diseases such as "osteoarthrosis," as recited in the present claims.

Barbier is strictly concerned with bone <u>repair</u>, the only working "repair" example (Barbier, Example 1, column 4) provides results from an animal model of a fracture, i.e., Barbier (column 4, lines 31-32) teaches: "The principle of hemiosteotomy is to produce an incomplete fracture line." The teaching of Barbier is, thus, limited to repair bone damages, such as from a fracture.

First, it is noted that fractures are specifically excluded from the scope of the present claims: the presently amended claim 1 refers to a process for treating lameness caused by osteoarthrosis in an animal not suffering from fracture. Consequently, the skilled person would not have considered Barbier to provide the claimed process of treatment.

Further, osteoarthrosis primarily concerns joint disease; additionally, the bone component of osteoarthrosis comprises sclerosis and osteophytes (this was previously presented during the interview of June 2006). These lesions are thus completely distinct from fractures. As a result, the
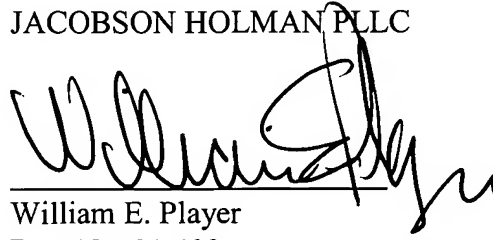
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skilled person would not have considered the teaching of Barbier, to provide a process of treatment

of osteoarthrosis in animal as presently claimed. Applicants thus submit that Barbier would not have

been considered by the skilled person, as alleged in the statement of rejection.

Favorable action is requested.

Respectfully submitted,

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GENETICS OF ENDOCRINE DISEASE

The Genetics of Paget's Disease of the Bone

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In 1877, Sir James Paget first described the localized skeletal disease that is now known as Paget's disease of the bone (PDB) (1). The disease is primarily a disorder of the osteoclast with marked increase in bone resorption, followed by abundant new bone formation. In this report, we present a brief overview of the genetics of PDB; a complete review was recently published by Singer and Leach (2).

PDB has a familial tendency (3–5), suggesting that there is a genetic predisposition. In a study of the frequency and characteristics of the familial aggregation of PDB in Spain, Morales-Piga *et al.* (6) found that 40% of their index cases had at least one first-degree relative affected with PDB. In the pedigrees they reported, PDB seemed to be transmitted through either parent, suggesting an autosomal dominant mode of inheritance.

Siris (7) conducted an epidemiological study of PDB in the United States, using questionnaires completed by 864 patients with physician-diagnosed PDB, and compared these results to 500 controls of similar age. A history of PDB was noted in a first-degree relative in 12% of the patients, compared with only 2% of controls. The risk of first-degree relatives of a pagetic patient developing PDB was seven times greater than for an individual without an affected relative. The cumulative risk for developing PDB up to age 90 for a first-degree relative of a patient was 9% compared with 2% in individuals with unaffected relatives. This is good evidence that a gene, or genes, plays a role in the acquisition of the disease.

Histocompatibility leukocyte antigen (HLA) association studies

The role of the major HLA loci on chromosome 6 in many human diseases makes it a logical starting point for studying the genetics of PDB. Several studies since 1975 have examined the possibility that there is an association between HLA and PDB (8–12). Although HLA typing of class I antigens

revealed no significant associations, significant associations were observed between class II antigens and PDB in two studies (11, 12). An increased incidence of HLA-DQW1 and HLA-DR2 was found in a preliminary report of 53 patients in Los Angeles (11). A second study of 25 Ashkenazi Jews in Israel revealed an increased incidence of HLA-DR2 in this population (12).

Family studies

Although multiple families have been reported with PDB, the average number of affected individuals in these families is three. Thus, these families are not sufficiently large for performing classical linkage analysis. However, there have been a few larger kindreds that have been used for linkage analysis. As reviewed below, analysis has identified two loci, one on chromosome 6 and one on chromosome 18, as susceptibility loci for PDB. Both loci seem to play a role in PDB, and there is evidence from other studies that there are other loci yet to be identified.

HLA linkage studies

The first linkage studies used the HLA loci because of their highly polymorphic nature. In a study conducted in New York City (13), three families with 29 informative children (all over the age of 45) were used. These kindreds were typed at the HLA-A, -B, and -C loci. Using haplotype data, the investigators obtained a maximum LOD (the logarithm of the odds ratio of a particular locus being linked or not linked to the disease locus) score of 2.44 with 11% recombination. Because a LOD score of 3 is considered linkage, these data were only deemed "suggestive." In a second study performed in New Zealand (14), two additional families were identified that seemed to segregate the disease in an autosomal dominant fashion. Each family had four affected members. These families were genotyped for the HLA loci. Although significant linkage was not obtained with this study, the combined linkage analysis from both studies resulted in a maximum LOD score of 3.69 with 10% recombination. The combined data were considered sufficient for establishing a predisposition locus for PDB on chromosome 6 near the HLA loci. Two other family studies have been published that explored the linkage of the HLA loci with PDB (15, 16). Both

studies reported no linkage between the HLA loci and a PDB predisposition gene. However, neither family was large enough to exclude linkage, and the relevance of these observations is unclear.

Chromosome 18 linkage studies

Familial expansile osteolysis (FEO) is a rare bone dysplasia, which is transmitted as an autosomal dominant trait (17) in a large kindred in Northern Ireland and a kindred in the United States. FEO shares some features with PDB. The bone lesions appear similar to early pagetic osteolytic lesions, although they occur at a much earlier age; also, these lesions never become sclerotic. Interestingly, osteoclasts from these patients contain paramyxoviral-like nuclear inclusions, which have also been reported in PDB (18). These results supported the hypothesis that FEO may be an allelic variant of PDB.

Hughes et al. (19) have used genetic linkage analysis to localize FEO to chromosome 18q. The disease shows a tight linkage with several polymorphic markers on chromosome 18q, with a maximum LOD score of 11.53 and no recombination. To further test the hypothesis that FEO is an allelic variant of PDB, linkage analysis has been performed between chromosome 18 markers and PDB disease kindreds.

The first published linkage study with chromosome 18 markers was performed by Cody et al. (20), using one large pedigree. The pedigree is presented in Fig. 1. In this study, 16 DNA samples were evaluated with a total of 12 markers

from 18q. The maximum LOD score of 3.44 was obtained with genetic marker D18S42 with no recombination. This marker lies in the same region of chromosome 18q that is linked to FEO. Thus, this study supports the hypothesis that FEO and PDB result from mutations in the same locus or tightly linked loci.

The following year, Haslam et al. (21) reported a study of eight families with familial PDB. Using seven polymorphic markers, they obtained a maximum LOD score of 2.97 with marker D18S42 with 5% recombination. Statistically, it seemed that the families were genetically heterogeneous. Only five of the eight families had positive LOD scores in this region, whereas the remainder seemed to be linked to another locus. There is also a preliminary report of a French pedigree that is clearly linked to 18q (22). This pedigree was analyzed with 12 chromosome 18 markers and gave a maximum LOD score of 3.10 with marker D18S68 at 1% recombination.

There have been several recent reports of families with PDB that do not seem to be linked to chromosome 18 (21, 23, 24). The most recent family was sufficiently large to exclude linkage across the entire interval (23). (A LOD score of −2 is needed to exclude linkage.) Unfortunately, the majority of the nonchromosome 18-linked families have not been evaluated with markers from chromosome 6. Until positive LOD scores are obtained with these "unlinked" families, it is difficult to estimate the number of predisposition loci for PDB.
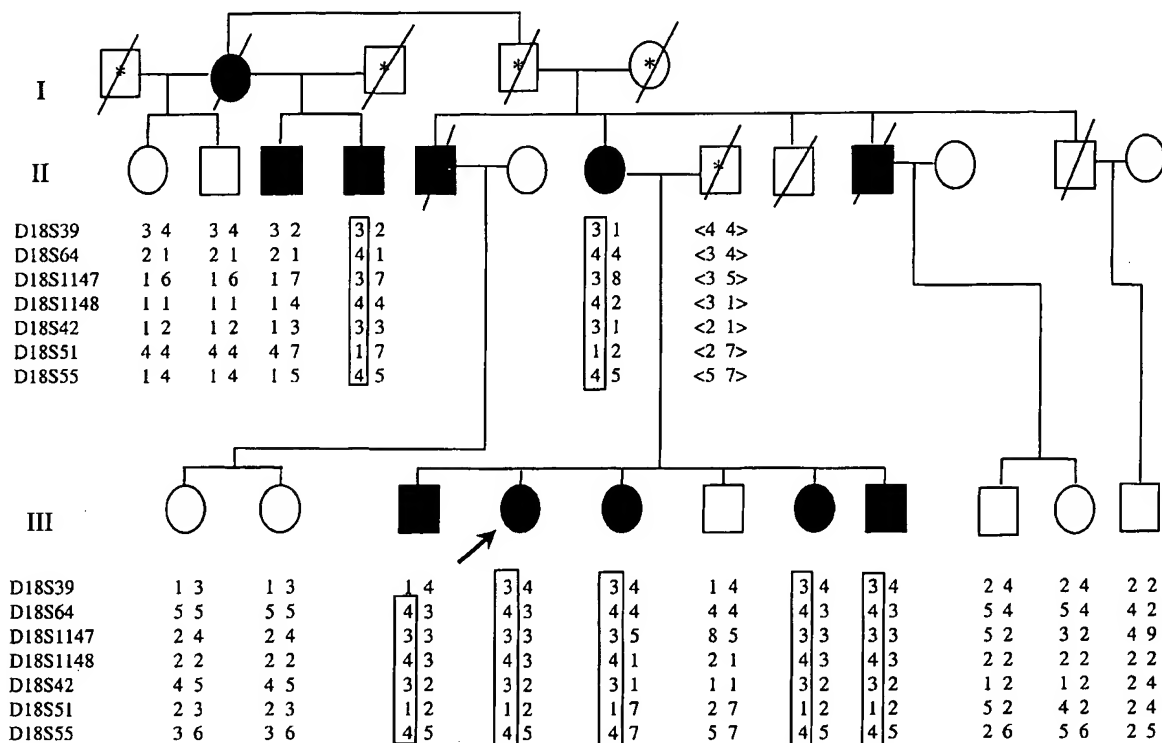

FIG. 1. Pedigree of kindred with PDB that is linked to the loci on chromosome 18q. Black symbols represent affected individuals. An asterisk indicates those individuals for whom the phenotype is unknown. The disease haplotype is indicated by a rectangle. [Adapted from Cody et al. (20).]

Role of receptor activator of nuclear factor κB (RANK) in FEO

RANK ligand (RANKL) is a newly described member of the tumor necrosis factor (TNF) family that has been identified as a critical osteoclastogenic factor (25–27). RANKL is expressed on marrow stromal cells and osteoblasts and seems to mediate the effects of most osteoclastogenic factors. *In vivo* studies have demonstrated that mice lacking RANKL develop severe osteopetrosis (28). Factors such as 1,25-$(OH)_2D_3$, interleukin 1, interleukin 11, and prostaglandin E_2 seem to induce osteoclast formation indirectly by up-regulating RANKL expression on marrow stromal cells (29). These data suggest that RANKL may be the common mediator for the effects of most osteotropic factors on osteoclast formation.

The receptor for RANKL, RANK, is a member of the TNF receptor family and interacts with TNF receptor-associated factor 2 and translocates it to the nucleus to induce nuclear factor κB signaling. RANK is expressed on osteoclast precursors and osteoclasts, and overexpression of RANK can induce nuclear factor kB signaling in the absence of RANKL (30). Furthermore, deletion of RANK by homologous recombination results in osteopetrosis (31). These data confirm the critical role of RANK/RANKL in osteoclastogenesis.

Earlier this year, Hughes *et al.* (32) identified the FEO gene on chromosome 18. Using mutational analysis, they identified gene mutations in three different FEO families. Interestingly, all three families carried the identical mutation. The mutation was an 18-bp in-frame insertion in the signal peptide sequence of the RANK gene (also known as TNFRSF11A). The 18-bp insertion was the result of a tandem duplication of bases 84 through 101 in exon 1. The mutation resulted in stabilization of the RANK protein, which in turn increased RANK signaling (32). As noted above, this increased RANK signaling could result in increased osteoclast formation, although this has not been proven.

Role of RANK in PDB

With the identification of the mutated gene in FEO on chromosome 18, it is now possible to test the hypothesis that FEO and PDB are the result of mutations in the same gene. Hughes *et al.* (32) screened 90 sporadic PDB patients and found none with this 18-bp insertion mutation. They also performed mutation screening in members of four PDB families who had evidence of linkage to 18q. All four of these families had been used in the linkage study by Haslam *et al.* (21). In one of these families, a slightly larger duplication involving bases 75 through 101 was observed in exon 1 (32). The PDB disease family with the mutation in RANK was of Japanese ancestry. This mutation segregated with the disease in this family. No other mutations were observed in the other three PDB families.

In the PDB family with the RANK mutation, most of the affected individuals presented in their teens and early twenties with bone pain or deformity. In addition, all the patients were described as having dental problems and several had hearing impairment. This clinical description begs the question whether this family has PDB *vs.* a mild version of FEO. It is uncommon in PDB to have tooth loss, but this seems to

be almost universal for FEO (33). In addition, hearing loss in PDB usually occurs later in life and is associated with the thickening of the skull, whereas hearing loss associated with FEO occurs early (33). Unfortunately, clinical information was not provided in the publication by Hughes *et al.* (32); thus, it is difficult to conclude that RANK mutations are associated with familial PDB, although it does not seem to play a major role in sporadic PDB.

To date, there have been two large families that demonstrated significant linkage to chromosome 18 (20, 22). It will be important to thoroughly evaluate the RANK gene in these families before making a conclusion concerning the role of RANK in familial PDB. The fact that the other families first described by Haslam *et al.* (21) did not show mutations in the RANK gene may be explained two different ways. First, the families may actually be linked to another locus. Clearly, there is more than one PDB predisposition gene, and these families are relatively small. Thus, linkage using these families can only identify a large region that carries the predisposition gene. It is possible that these families are truly linked to chromosome 18 and that the responsible gene is near the RANK locus, or that they are linked to another chromosome. Alternatively, the "mutation" in the RANK gene could be a promoter "mutation" that slightly increases the gene expression. Such a "mutation" may be only a polymorphism, and it may be difficult to demonstrate functional significance. Based on the data for the insertion mutation and the role of RANK in osteoclast differentiation, one would expect that any alterations in the RANK gene causing PDB would demonstrate increased RANK signaling.

PDB and osteosarcoma

In the majority of patients, PDB is asymptomatic (34). The 5–10% of patients with symptoms have bone pain with a wide range of complications, including increased fractures, deafness, and neurological findings (35). The most devastating complication of PDB is malignant transformation of the bone. Although these transformations are rare, occurring in less than 1% of patients, they contribute significantly to the morbidity and mortality of the disease. There are various forms of malignant transformation associated with PDB, but the most frequent is osteosarcoma. Osteosarcoma, like most cancers, is believed to result from a series of genetic alterations that transform the osteoblast to a malignant state. There is interesting evidence to imply that there is a molecular connection between osteosarcoma and PDB (36).

Loss of heterozygosity (LOH) mapping is a method used to identify tumor suppressor loci in cancer (37). Yamaguchi *et al.* (38) have used LOH mapping to examine osteosarcomas. They found high-frequency LOH on 3q, 13q, 17p, and 18q, suggesting that these chromosome arms contain tumor suppressor genes important in the development of osteosarcoma. Nellisery *et al.* (36) have further refined the location of this tumor suppressor gene between genetic markers D18S60 and D18S42 (see Fig. 2). This is the same region that codes the FEO and the familial PDB genes. This has led us to the hypothesis that there is a link between the predisposition gene for PDB and the tumor suppressor gene for osteosarcoma on chromosome 18q. Interestingly, seven of seven os-
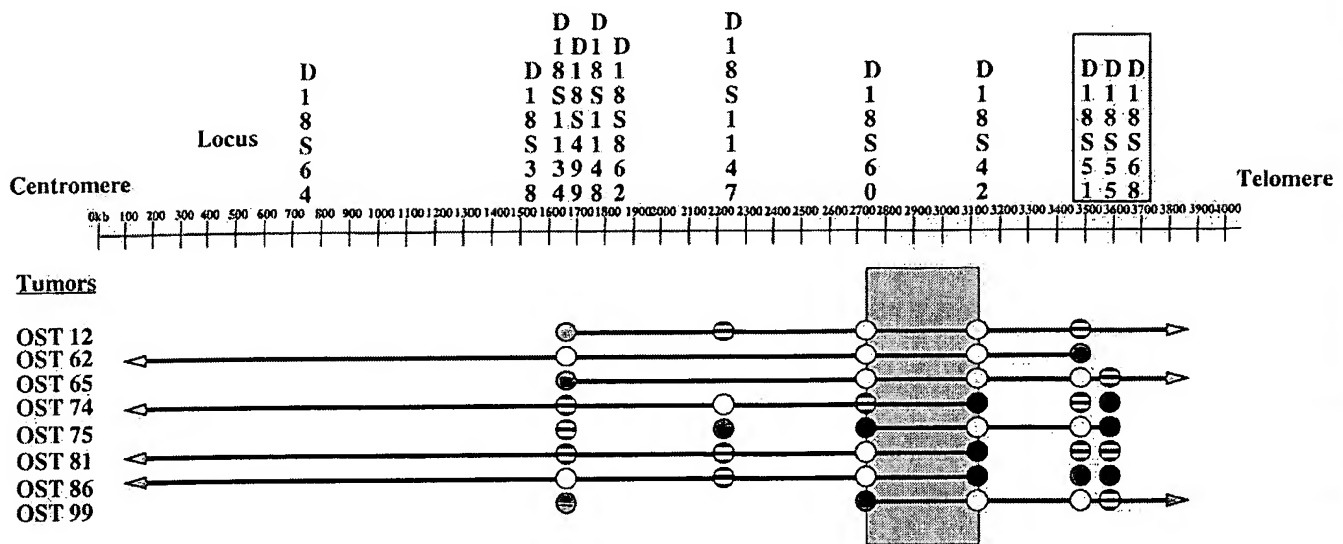
FIG. 2. Map of the minimal region of LOH on chromosome 18q in osteosarcoma tumors. The polymorphic loci and their estimated physical distances are shown across the *top*. Note that D18S51, D18S55, and D18S68 were not ordered in the contig. Below the map is the information gained from the LOH studies of each tumor. ⊘, No LOH; ○, LOH. Uninformative markers have a *dash*. The *arrows* depict the region and the direction of LOH for each tumor. The minimal region of LOH in the sporadic osteosarcomas is denoted by the *large shaded rectangle*. [Adapted from Nellissery *et al.* (36).]

teosarcomas from PDB patients showed LOH in this region (36). However, it is unclear, at present, whether the PDB predisposition gene and the osteosarcoma tumor suppressor gene are one and the same gene or two tightly linked genes on chromosome 18q.

Molecular analysis by Hughes *et al.* (32) localized the RANK gene between markers D18S64 and D18S51, which was the same interval known to contain the FEO locus. Further mapping in our laboratory has demonstrated that the RANK gene lies between markers D18S60 and D18S42 (R. J. Leach, unpublished data), which is the critical region for the putative osteosarcoma tumor suppressor gene (see Fig. 2). These data suggest that the role of RANK in osteosarcoma needs to be thoroughly analyzed.

PDB and paramyxovirus

In addition to a genetic predisposition for PDB, many studies have suggested a potential viral etiology for PDB as well (39), because pagetic osteoclasts contain paramyxoviral-like nuclear inclusions. Although the presence of a virus in pagetic osteoclasts is still controversial, the majority of studies have supported either measles virus or canine distemper virus as the paramyxovirus present in pagetic osteoclasts and their precursors. It is possible that the combination of a genetic predisposition to PDB and chronic infection of osteoclast precursors by paramyxovirus may be required to express the pagetic phenotype in cells of the osteoclast lineage. However, until a virus is isolated or a paramyxoviral gene can be shown to induce PDB in an animal model, the role of the virus in the pathogenesis of PDB is still unclear.

Conclusion

PDB disease clearly has a hereditary component. Family studies demonstrate that there is more than one predispo-

sition gene for PDB. The mapping of osteosarcoma tumor suppressor and a PDB predisposition locus on 18q makes this region of the genome of particular interest, and a region of future study. Until more extensive linkage analyses are performed on larger kindreds with classic PDB, the other susceptibility loci for PDB will remain elusive. The identification of PDB predisposition genes will increase our understanding of the pathogenesis of this disorder and could lead to alternate treatment strategies. In addition, the identification of these genes will be useful for understanding bone biology and the molecular basis of the alterations that give rise to PDB.

References

1. Paget J. 1877 On a form of chronic inflammation of bones (osteitis deformans). Med Chir Trans. 60:37–64.
2. Singer FR, Leach RJ. 2000 Genetics of Paget's disease of bone. In: Econs MJ, ed. The genetics of osteoporosis and metabolic bone disease. Totowa, NJ: Humana Press; 309–318.
3. Jacobs TP, Michelsen J, Polay JS, D'Adamo AC, Canfield RE. 1979 Giant cell tumors in Paget disease of bone: familial and geographic clustering. Cancer. 44:742–747.
4. Sofaer JA, Holloway SM, Emery A. 1983 A family study of Paget disease of bone. J Epidemiol Community Health. 37:226–231.
5. Mirra JM. 1986 CPC case: Paget disease and giant cell tumor. N Engl J Med. 314:105–113.
6. Morales-Piga AA, Rey-Rey JS, Corres-Gonzales J, Garcia-Sagredo JM, Lopez-Abente G. 1995 Frequency and characteristics of familial aggregation in Paget disease of bone. J Bone Miner Res. 10:663–670.
7. Siris ES. 1994 Epidemiological aspects of Paget disease: family history and relationship to other medical conditions. Semin Arthritis Rheum. 23:222–225.
8. Roux H, Mercier P, Maestracci D, Eisinger J, Recordier AM. 1975 HL-A et maladie de Paget. Rev Rhum Mal Osteoartic. 42:661–662.
9. Cullen P, Russel GG, Walton RJ, Whiteley J. 1976 Frequencies of HLA-A and HLA-B histocompatibility antigens in Paget's disease of bone. Tissue Antigens. 7:55–56.
10. Gordon MT, Cartwright EJ, Mercer S, Anderson DC, Sharpe PT. 1994 HLA polymorphisms in Paget's disease of bone. Semin Arthritis Rheum. 23:229.
11. Singer RF, Mills BG, Park MS, Takemura S, Terasaki PI. 1985 Increased HLA-DQWI antigen pattern in Paget's disease of bone. Clin Res. 33:574A.
12. Foldes J, Shamir S, Brautbor C, Schermann L, Menczel J. 1991 HLA-D antigens and Paget's disease of bone. Clin Orthop. 266:301–303.

13. Fotino M, Haymovits A, Falk CT. 1977 Evidence for linkage between HLA and Paget's disease. Transplant Proc. 9:1867–1868.
14. Tilyard MW, Gardner RJM, Milligan L, Cleary TA, Stewart RDH. 1982 A probable linkage between familial Paget's disease and HLA loci. Aust NZ J Med. 12:498–500.
15. Moore SB, Hoffman DL. 1988 Absence of HLA linkage in a family with osteitis deformans (Paget's disease of bone). Tissue Antigens. 31:69–70.
16. Kim GS, Kim SH, Cho JK, et al. 1997 Paget bone disease involving young adults in 3 generations of a Korean family. Medicine. 76:157–169.
17. Wallace RG, Barr RJ, Osterberg PH, Mollan RA. 1989 Familial expansile osteolysis. Clin Orthop. 248:265–277.
18. Dickson GR, Shirodira PV, Kanis JA, Beneton MN, Carr KE, Mollan RAB. 1991 Familial expansile osteolysis: a morphological, histomorphometric and serological study. Bone. 12:331–338.
19. Hughes AE, Shearman AM, Weber JL, et al. 1994 Genetic linkage of familial expansile osteolysis to chromosome 18q. Hum Mol Genet. 3:359–361.
20. Cody JD, Singer FR, Roodman GD, et al. 1997 Genetic linkage of Paget disease of the bone to chromosome 18q. Am J Hum Genet. 61:1117–1112.
21. Haslam SI, Van Hul W, Morales-Piga A, et al. 1998 Paget's disease of bone: evidence for a susceptibility locus on chromosome 18q and for genetic heterogeneity. J Bone Miner Res. 13:911–917.
22. Lucotte G. 1999 Genetic linkage of Paget's disease to chromosome 18q in a French pedigree (Abstract). Bone. 24:31S.
23. Nance MA, Nuttall FQ, Econs MJ, et al. 2000 Heterogeneity of Paget disease of the bone. Am J Med Genet. 92:303–307.
24. Hocking L, Slee F, Haslam SI, et al. 2000 Familial Paget's disease of bone: Pattern of inheritance and frequency of linkage to chromosome 18q. Bone. 26:577–580.
25. Lacey DL, Timms E, Tan HL, et al. 1998 Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 93:165–176.
26. Yasuda H, Shima N, Nakagawa N, et al. 1998 Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA. 95:3597–3602.

27. Hofbauer LC, Heufelder AE. 1998 Osteoprotegerin and its cognate ligand: a new paradigm of osteoclastogenesis. Eur J Endocrinol. 139:152–154.
28. Kong YY, Yoshida H, Sarosi I, et al. 1999 OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature. 397:315–323.
29. Yasuda H, Shima N, Nakagawa N, et al. 1998 Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Endocrinology. 139:1329–1337.
30. Anderson DM, Maraskovsky E, Billingsley WL, et al. 1997 A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature. 390:175–179.
31. Dougall WC, Glaccum M, Charrier K, et al. 1999 RANK is essential for osteoclast and lymph node development. Genes Dev. 13:2412–2424.
32. Hughes AE, Ralston ST, Marken J, et al. 2000 Mutations in TNFRSF11A affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet. 24:45–49.
33. Hughes AE, Barr RJ. 1996 Familial expansile osteolysis: a genetic model of Paget's disease. In: Sharpe PT, ed. The molecular biology of Paget's disease. Austin, TX: R.G. Landes Company; 179–200.
34. Dalinka MK, Aronchick JM, Haddack Jr JG. 1983 Paget's disease. Orthop Clin North Am. 14:3–19.
35. Cawley MI. 1983 Complication of Paget's disease of bone. Gerontology. 29:276–287.
36. Nellissery MJ, Padalecki SS, Brkanac Z, et al. 1998 Evidence for a novel osteosarcoma tumor-suppressor gene in the chromosome 18 region genetically linked with Paget disease of bone. Am J Hum Genet. 63:817–824.
37. Hansen MF, Cavenee WK. 1988 Tumor suppressors: recessive mutations that lead to cancer. Cell. 53:173–174.
38. Yamaguchi T, Toguchida J, Yamamuro T, et al. 1992 Allelotype analysis in osteosarcomas: frequent allele loss on 3q, 13q, 17p, and 18q. Cancer Res. 52:2419–2423.
39. Singer FR, Roodman GD. 1996 Paget's disease. In: Bilezikian J, Raisz L, Rodan G, eds. Principles of bone biology. San Diego: Academic Press; 969–977.

Paget's disease of bone: Symptoms, signs and morbidity

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I. Introduction

Paget's disease of bone, named after Sir James Paget, who first accurately observed and described its clinical manifestations in 1877, is a focal disorder of bone remodelling in which bone becomes structurally chaotic, less compact, more vascular and more prone to deformity and fracture. Over the years, light has been shed on the aetiology, pathophysiology, epidemiology and management of Paget's disease of bone [1–12]. Little has been added, however, to the clinical features of the untreated disorder as originally described by Sir James Paget at the turn of the century [13].

...It begins in middle age or later, is very slow in progress, may continue many years without influence on the general health, and may give no other trouble than those which are due to changes of shape, size and direction of the diseased bones.

... The skull became gradually larger, so that nearly every year, for many years, his hat, and the helmet that he wore as a member of a Yeomanry Corps needed to be enlarged....

... The length of the spine seemed lessened, and from a height of six feet one inch, he sank to about 5 feet nine inches.... The arms appeared unnaturally long... the hands hung low down by the thighs and in front of them. Altogether the attitude in standing looked simian, strangely in contrast with the large head...

... The left tibia had become larger, and had a well marked anterior curve as if lengthened, while its ends were held in place by their attachments to the unchanged fibula....

Sir James Paget (1877)

II. Pathophysiology

Paget's disease of bone is characterised by regional increases in bone turnover. Although geographic, ethnic and genetic factors may play a contributory role to the pathophysiology of the disorder, it is now widely believed that the pathological lesion could be the late manifestation of a slow virus infection resulting in a local, often multifocal abnormality in osteoclast morphology, number and function. The

distribution of the lesions and their strict localisation within the boundaries of the part of the skeleton involved tend to support the notion of a possible haematogenous route for the acquisition of the initial insult, analogous to the distribution of bony lesions in haematogenous bacterial osteomyelitis. The demonstration of nuclear inclusion bodies possibly belonging to the paramyxovirus family, in osteoclasts at pathological sites appears to support the hypothesis of a viral aetiology for the abnormalities observed, although the putative organism has not yet been definitely identified. The resulting regional increase in bone resorption is characteristically associated with a proportional increase in bone formation as a result of greatly increased numbers of essentially normal osteoblasts. A ten-fold increase in bone turnover may be observed in involved bones, with up to 100% of the bone surface occupied by active remodelling events. The rapid rates of bone formation result in the 'mosaic pattern' chaotic deposition of woven bone, lacking the structural organisation of lamellar bone, occupying more space, and more prone to distortion by mechanical forces. Excessive fibrous tissue and blood vessels infiltrate the adjacent bone marrow, resulting in highly vascular lesions, particularly in the active stages of the disease. Following treatment, lamellar bone deposition resumes, and the vascularity of the lesions decreases.

III. Clinical features

The disease is most commonly diagnosed after the age of 50 years, and its incidence rises with increasing age. There is a slight male preponderance, and there may be a difference in skeletal distribution between men and women, with the skull and face appearing to be more frequently affected in women compared to a male preponderance for other localisations. Skeletal involvement is heterogeneous, asymmetrical in distribution, and best evaluated by bone scintigraphy. The most common sites affected are the pelvis (in two-thirds of patients), the femur, spine, skull and tibia. The upper extremities are much less frequently affected. The disease may be monostotic (in about 25% of patients) or more commonly polyostotic, involving two or more bones.

The onset of the disease is insidious, and symptoms may vary from minimal to severely disabling, depending on areas of involvement, adjacent structures and activity of the disease. At presentation, 30% of patients will have had symptoms for 10 years or more, but only a third of the lesions is usually symptomatic. A significant percentage of patients is thus asymptomatic at presentation, and the diagnosis is incidentally established clinically (deformity, particularly of a long bone), biochemically (elevated serum alkaline phosphatase activity on biochemical screening for another indication), or radiologically (typical disordered structure of the pagetic bone). Clinical features of the disorder depend on the localisation, activity and extent of the pagetic lesions.

I. Bone pain

Bone pain is the most frequent presenting symptom in Paget's disease of bone. The pain is usually dull, non-specific, but may be boring or nagging in nature. Pain is unrelated to exercise or weight-bearing and bears little relationship to radiographic appearances, although some authors suggest that lesions may be more painful when sclerotic.

Pain is more common in lesions of the long bones. In the presence of microfractures, which may not be necessarily visualised radiographically, it may become sharper and more localised. Headaches or band-like tightness across the forehead are common when Paget's disease is localised to the skull

Bone pain requires careful evaluation in Paget's disease of bone, as its aetiology is usually multifactorial. Bone pain may be primarily related to the pathological pagetic process, or may arise as a result of complications at the site of a lesion or in adjacent structures. Primarily related to the pagetic process is an increase in bone vascularity in active lesions [14], which may lead to a rise in intramedullary pressure and pain. Pain has indeed been observed to be more common in patients in whom an increase in skin temperature can be demonstrated. Bone pain could also be due to stretching of the periosteum by the increased bone volume due to its disordered deposition during disease activity. Reports of symptomatic relief of pain following decompression by the drilling of holes at the site of the bony lesion possibly substantiate this view. Localised pain may also be due to hyperaemia of the marrow cavity leading to stimulation of adjacent somatic nerve endings, to secondary degenerative changes in adjacent or contralateral joints to a pagetic lesion, or to microfractures in a weight-bearing area.

The pagetic process is often associated with osteoarthritic changes due to unnatural stresses on a weight-bearing joint as a result of deformities [15, 16]. The pelvis is commonly affected in Paget's disease of bone, and the hip joints are thus frequently involved in the disease process, with the degenerative changes occurring on the same side as the pagetic lesion or on the contralateral non-affected side due to altered weight-bearing.

Pain can also occur as a result of neurological compression syndromes, vascular steal syndromes or when the disease is complicated by the development of osteosarcoma.

The vertebrae represent a common site of involvement in Paget's disease of bone, and the lumbar and sacral vertebrae are the ones most frequently affected. The most common presenting symptom is back pain, although the majority of patients with uncomplicated spinal localisation of Paget's disease are asymptomatic. The expanded vertebrae are usually incidentally visualised radiologically or on bone scintigraphy. When present, back pain could be non-specific due to the active pagetic process, or may occur as a result of vertebral compression fractures, spinal stenosis (resulting in radicular pain), a vascular steal syndrome or, commonly, secondary degenerative changes.
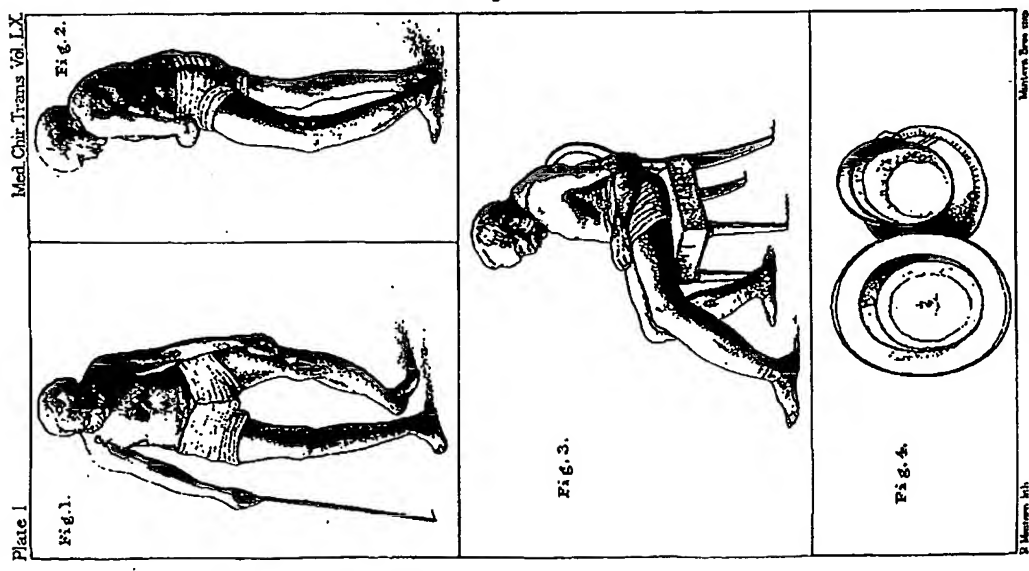
Plate 1

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Fig. 1. Reproduction of lithographies, from Sir James Paget's original 1877 publication [13].

2. Deformity

Deformity is the presenting feature in some 20% of patients with Paget's disease of bone and occurs as a result of structural weakness of the pathological bone. The long bones are particularly liable to deformity with resulting anterior (tibia) or lateral (femur and humerus) bowing, depending on mechanical stress and lines of least resistance. Bowing of the femur is often associated with a coxa vara deformity, resulting in external rotation of the lower leg. Lengthening of the affected limb may result in gait abnormalities, and clinically severe secondary arthritis may occur as a result of abnormal mechanical stresses. Fractures may also occur at the sites of maximum bowing.

In the pelvis, involved in about two-thirds of patients, softening of the acetabulum may result in marked deformity, with the femoral head invaginating the pelvic rim, "protrusio acetabuli". Acetabular protrusion may lead to severe hip joint dysfunction and secondary degenerative changes.

Vertebral crush fractures are associated with loss of height and increased curvature of the spine. As a result of spinal bowing, the limbs appear disproportionately long, giving the patient a simian appearance (Figure 1). Occasionally, expanding vertebral bodies may encroach on intervertebral foramina. This may result in interference to the blood supply of the spinal cord and a 'steal syndrome', or in narrowing of the spinal canal and neurological compression features.

The skull is involved in about one-third of patients with Paget's disease of bone, usually associated with more extensive disease elsewhere. Women appear to be more frequently affected than men. Involvement of the vault of the skull results in an increase in the size of the head, which may become triangular in shape. Shortening of the neck due to basilar invagination is observed with involvement of the base of the skull, and the head appears to be sinking onto the shoulders. Radiologically, Paget's disease of the skull may start as a well delineated rarefied area, "osteoporosis circumscripta", involving mostly the frontal or occipital bones, but which may spread to involve the whole of the skull vault. Sclerotic lesions of the skull are, nevertheless, twice as frequent as osteoporotic ones. Involvement of the facial bones is rare but may result in significant cosmetic and dental problems, and marked deformity of the maxillary bones may interfere with speech, mastication and deglutition and may result in loosening of the teeth [17].

3. Local changes

The skin overlying an active pagetic lesion may be warm to palpation, partly due to increased skeletal blood flow [14], but also due to a local increase in metabolic rate and bone turnover with compensatory vasodilatation in the overlying tissues to dissipate the excessive heat generated. The increase in skin temperature is most commonly appreciated in the distal extremities, and the local increase in vascularity is occasionally associated with a bruit, which can be heard on auscultation of the affected site. The increased vascularity of an active pagetic lesion may also compromise the overlying skin blood flow and result in atrophy and the development of slow-healing ulcers.

IV. Biochemical features

The hallmark of active Paget's disease of bone is the balanced increase in bone resorption and formation, traditionally assessed by estimating the urinary excretion of hydroxyproline, the most widely used index of bone collagen degradation, and serum alkaline phosphatase activity, an index of the number of functioning osteoblasts and hence bone formation. Both indices are closely correlated with bone turnover and with the extent and activity of Paget's disease as judged radiologically, scintigraphically and by calcium tracer techniques. These indices may, however lie within the normal range in 10–20% of patients with localised monostotic disease, or be inordinately elevated in the case of skull involvement.

Collagen is the major protein of bone, and about 50% of the total urinary hydroxyproline is derived from this source. Dietary collagen is a significant source of hydroxyproline, and dietary restriction of meat and dairy products is required for the accurate interpretation of this index. The need for dietary restriction can, however, be obviated with the use of measurements done on early morning specimens taken after an overnight fast, with values expressed as a ratio of hydroxyproline to creatinine excretion. In Paget's disease of bone, a significant relationship has indeed been demonstrated between fasting hydroxyproline to creatinine ratio and corresponding 24-h urinary excretion of hydroxyproline, and the use of early morning samples has been advocated in the monitoring of response to treatment [18].

A well-characterised biochemical sequence of events is observed in urinary hydroxyproline excretion and serum alkaline phosphatase activity following treatment with inhibitors of osteoclast-mediated bone resorption such as the bisphosphonates. Bone resorption is suppressed early (days), as judged by a decrease in the urinary excretion of hydroxyproline, and a secondary decrease in bone formation follows (weeks), as judged by a decrease in serum alkaline phosphatase activity. The rate and the degree of suppression of bone resorption vary with the potency of the bisphosphonate used, and a complete clinical and biochemical response could be achieved in over 90% of patients with the use of the newer, more potent bisphosphonates. Normalisation of the urinary excretion of hydroxyproline and serum activity of alkaline phosphatase to well within the normal range is a good predictor of a long-lasting remission, whichever the bisphosphonate used and should be aimed at, when devising treatment regimens. These indices should be regularly monitored after remission is achieved (once or twice a year), with an increase in either heralding a relapse in disease activity.

In the event of failure of suppression of alkaline phosphatase activity with the use of adequate suppressive doses of bisphosphonates, or in the case of a rapid relapse after an initial satisfactory response as indicated by an early increase in the enzyme concentrations, malignant transformation of a lesion should be excluded, particularly if associated with persisting symptoms.

Other indices of bone resorption and formation have been examined in the management of Paget's disease of bone. Pyridinoline and deoxypyridinoline are non-reducible intermolecular crosslinks between adjacent mature collagen chains, present only in extracellular collagen fibrils and released in the circulation during

the degradation of bone and cartilage. The urinary excretion of these collagen degradation products forms useful and specific indices of bone resorption in Paget's disease of bone. It has been proposed that the urinary excretion of these crosslinks may be a more sensitive index of collagen degradation than hydroxyproline in Paget's disease of bone [19], particularly as they are not affected by dietary gelatin intake. Contrary to the case with the urinary excretion of hydroxyproline, however, data are not yet available on the relationship of these indices to the extent or activity of the disease process. Moreover, recent studies have also demonstrated that these new indices do not appear to offer any advantage over the traditional determination of the urinary excretion of hydroxyproline in the monitoring of response to treatment with inhibitors of bone resorption [20].

Procollagen, the immediate precursor to the α-chain of the final collagen molecule, is a larger protein molecule than collagen, containing additional sequences at both ends. Procollagen is synthesised intracellularly in osteoblasts and is cleaved from the final collagen molecule by specific proteases before it is assembled into fibrils and stabilised by cross-links. The cleaved fragments from the carboxy-terminal pro-peptide of type I procollagen (PICP) are liberated into the circulation where they can be measured by radioimmunoassay. Circulating procollagen peptides are thought to be directly related to the number of collagen molecules formed and are therefore used to quantify type I collagen synthesis and hence bone formation. PICP have indeed been shown to correlate significantly with histologically derived bone formation rates in patients with various disorders of bone metabolism and have been shown to be useful in the clinical monitoring of Paget's disease of bone [21].

Bone formation could also be indirectly assessed by the estimation of serum concentrations of fragments of matrix proteins released in the circulation during the process of bone formation such as osteocalcin. Osteocalcin is the most abundant non-collagenous protein in bone and is synthesised exclusively by osteoblasts. In untreated Paget's disease of bone, osteocalcin concentrations have, however, been found to be normal in about 50% of patients in whom serum alkaline phosphatase activity was increased. In the course of treatment with inhibitors of bone resorption, changes in serum concentrations of osteocalcin do not parallel those of the two traditionally used indices of bone turnover, and an initial increase may be observed after the start of treatment [22]. The use of osteocalcin as an index of bone turnover is thus not advocated in Paget's disease of bone, as it is less sensitive and specific than either urinary excretion of hydroxyproline or serum alkaline phosphatase activity in the assessment of disease activity or in the monitoring of its response to treatment.

Extracellular calcium homeostasis is usually maintained in Paget's disease of bone, although a number of patients may be hypercalciuric and may develop hypercalcaemia on immobilisation, particularly in the presence of extensive skeletal involvement. The risk of urolithiasis is increased in the presence of persistent hypercalciuria. An increase in parathyroid hormone concentrations can also be observed in up to 20% of patients in the presence of normal serum calcium concentrations, particularly in those patients with the greater skeletal involvement.

In the early phase of treatment with inhibitors of bone resorption, the suppression of bone resorption, in the presence of continuing bone formation, results in a decrease in the net efflux of calcium from the skeleton to the extracellular fluid. A fall in serum calcium follows, which stimulates PTH secretion. This in turn increases the renal tubular reabsorption of calcium and enhances intestinal absorption of calcium via stimulation of calcitriol synthesis by the kidney, thus restoring calcium homeostasis. These secondary metabolic effects are not noted with the use of etidronate, as the associated delay in mineralization, particularly with the use of high oral or intravenous doses, inhibits calcium accretion in bone and offsets any hypocalcaemic effect resulting from the inhibition of bone resorption. The unopposed effect of the bisphosphonate on renal tubular reabsorption of phosphate results in hyperphosphataemia, an effect which is dose-dependent in the case of etidronate and only seen very transiently with the use of other bisphosphonates, when it is usually abolished by a secondary increase in PTH secretion [23].

V Complications

Complications of Paget's disease of bone were also recognised and described by Sir James Paget in his original series of untreated patients whom he observed long-term.

> ... as he was riding and suddenly raised his arms, the bone broke near the shoulder....
> ... cancer appeared late in life, ... possibly not more than might have occurred in accidental coincidences, yet suggesting careful enquiry....

Untreated Paget's disease of bone may remain silent for years, and patients may present with complications including fractures [24], secondary degenerative changes in adjacent or distant joints [15, 16], neurological complications [25] or, more rarely, neoplastic changes [26, 27].

1. Fractures

Pagetic bone is deposited in a disorganised manner, so that the bone is mechanically weak, although increased in volume. Fractures are infrequent, occurring in only about 5% of cases. They represent, however, a serious complication of Paget's disease of bone. They occur most commonly in weight-bearing long bones, at the site of maximal mechanical stresses as a result of bowing, and may be fissure or complete fractures. Long-standing untreated disease resulting in a bowing deformity of a long bone is thus a significant predisposing factor.

Fractures may occur spontaneously or after minimal trauma, may be single or multiple and, in view of the increased vascularity of the lesions, may result in a substantial loss of blood. Fractures may be asymptomatic, but patients may also present with pain and tenderness, often heralding the development of a fissure fracture into a complete one. Fissure fractures may precede the development of a complete fracture by a few weeks.

Some 70–90% of pathological fractures occur in the femur, followed in frequency by fractures of the tibia and forearm. Vertebral compression fractures may also occur when the spine is involved.

Most fractures through pagetic bone heal normally, but union may be delayed, and refracture is common. Fractures of the femoral neck represent a particular management problem, as the bone may be too soft to provide support in the osteolytic phase, or too difficult to penetrate in the sclerotic phase. Deformity may also be so severe that alignment of the shaft may be difficult and internal fixation impossible. Fractures may also be complicated by hypercalcaemia as a result of enforced immobilisation. This may in turn be associated with hypercalciuria and an increased risk of nephrocalcinosis and nephrolithiasis. There is no evidence that fractures are associated with an increased risk of developing osteosarcoma.

2. Osteoarthritis

Osteoarthritis is a very frequent complication of Paget's disease of bone, occurring in joints adjacent to pagetic bone or on the contralateral side as a result of altered lines of mechanical stresses on weight-bearing surfaces. Clinically severe secondary arthritis occurs in a significant proportion of patients with Paget's disease affecting the pelvis. Patients present with pain and limitation of movement in the affected sites. Symptoms may significantly improve by controlling disease activity.

3. Neoplastic change

In Paget's disease of bone, neoplastic changes can occur at the site of any lesion, most commonly in the form of osteosarcoma. It is very difficult to estimate the incidence of this rare but most serious complication of the disorder. Its prevalence increases with age, peaking at the seventh decade, and the most common sites affected are the pelvis, long bones (humerus and femur) and skull. Contrary to primary osteosarcoma which mostly affects the metaphyseal region of bone, Paget's osteosarcoma can occur anywhere along the shaft of a long bone or at any other site affected. A worsening in pain at the site of a known lesion or a change in the pattern of pain may herald osteosarcomatous changes. A fracture may also be the first sign of malignant transformation of a lesion, in which case healing is often delayed. The bony changes may be associated with soft-tissue swelling and tenderness and dilatation of superficial veins. Bone destruction can be demonstrated radiologically in the form of osteolysis with ill-defined boundaries, and bone scintigraphy demonstrates decreased uptake.

The osteosarcomatous lesion is frequently multicentric, and its extreme vascularity increases the risk of haematogenous spread. In over 50% of cases, the anatomical site of the lesion is not amenable to radical amputation. The neoplastic change is also often diagnosed too late, when radical amputation may not alter the prognosis, which is thus very poor. Mean survival has been reported to vary between 7 and 14 months.

4. Neurological complications

Neurological complications may occur in Paget's disease of bone as a result of increased pressure on neurological structures from enlarging bones, or due to interference with the regional neuronal vascular supply.

Cranial nerve compression can occur in Paget's disease of the skull, as a result of narrowing of cranial nerve foramina. The most anatomically susceptible nerves are the optic, auditory and trigeminal nerves, leading, respectively, to progressive loss of vision, deafness and trigeminal neuralgia. Hearing impairment is common in Paget's of the skull and could be conductive, due to fixation of the bone ossicles in the middle ear, or neuronal, due to compression of the VIIIth cranial nerve in the auditory canal. Irreversible hearing loss may occur in 20% of the patients. The lower cranial nerves are less frequently affected. Direct compression of the brain stem in Paget's of the base of the skull may result in progressive confusion.

Paget's disease of the spine can result in nerve root compression, narrowing of the spinal canal or interference with the blood supply to the spinal cord, 'the steal syndrome'. Symptoms may resolve on adequate suppression of disease activity.

5. Cardiovascular complications

In Paget's disease of bone, heart failure is most commonly due to underlying cardiac disease. Rarely, increased vascularity in extensive lesions may result in high output cardiac failure [28]. This complication is seldom, if ever, seen nowadays.

6. Urolithiasis

Renal stones are present in about 5% of patients with Paget's disease of bone. Possible risk factors include hypercalciuria and hypercalcaemia, possibly resulting from immobilisation for concurrent illnesses. Serum uric acid is also often elevated.

References

1. Collins, D.H., Paget's disease of bone — Incidence and subclinical forms. Lancet 1956; ii: 51–57.
2. Nagant de Deuxchaisnes, C. and Krane, S.M., Paget's disease of bone: Clinical and metabolic observations. Medicine 1964; 43: 233–266.
3. Krane, S.M., Paget's disease of bone. Clin. Orthop. Rel. Res. 1977; 127: 24–36.
4. Singer, F.R., Schiller, A.L., Pyle, E.B. and Krane, S.M., Paget's disease of bone. In: Avioli, L. and Krane, S., eds, Metabolic Bone Disease. London: Academic Press, 1978; 489–575.
5. Altman, R.D. and Collins, B., Musculoskeletal manifestations of Paget's disease of bone. Arthritis Rheum. 1980; 23: 1121–1127.
6. Hamdy, R.C., Paget's disease of bone: Assessment and management. New York: Praeger, 1981.
7. Russell, R.G.G., Paget's disease. In: Nordin, B.E.C, ed, Metabolic bone and stone disease. Edinburgh: Churchill Livingstone, 1984; 190–233.
8. Harinck, H.I.J., Bijvoet, O.L.M., Vellenga, C.G.R.L., Blanksma, H.J. and Frijlink, W.B., Relation between signs and symptoms of Paget's disease of bone. Q. J. Med. 1986; 58: 133–151.
9. Meunier, P.J., Salson, C., Mathieu, L., Chapuy, M.C., Delmas, P., Alexandre, C. and Charhon, S., Skeletal distribution of Paget's disease. Clin. Orthop. Rel. Res. 1987; 217: 37–44.

10. Resnick, C.S., Paget's disease of bone: Current status and a look back to 1943 and earlier. Am. J. Radiol. 1988; 150: 249–256.
11. Bijvoet, O.L.M., Vellenga, C.J.L.R. and Harinck, H.I.J., Paget's disease of bone: Assessment, therapy, and secondary prevention. In: Kleerekoper, M. and Krane, S.M., eds, Clinical disorders of bone and mineral metabolism. New York: Liebert, 1989; 525–542.
12. Kanis, J.A., Pathophysiology and treatment of Paget's disease of bone. London: Martin Dunitz, 1991.
13. Paget, J., On a form of chronic inflammation of bones. Medico-Chirurgical Trans. 1877; 60: 37–63.
14. Wootton, R., Reeve, J., Spellacy, E. and Teltez-Yudilevich, M., Skeletal blood flow in Paget's disease of bone and its response to calcitonin therapy. Clin. Sci. Mol. Med. 1978; 54: 69–74.
15. Franck, W., Bress, N., Singer, F. and Krane, S., Rheumatic manifestations of Paget's disease of bone. Am. J. Med. 1974; 56: 592–603.
16. Winfield, J. and Stamp, T.C.B., Bone and joint symptoms in Paget's disease. Ann. Rheum. Dis. 1984; 43: 769–773.
17. Smith, B.J. and Eveson, J.W., Paget's disease of bone with particular reference to dentistry. J. Oral Pathol. 1981; 10: 233–247.
18. Russell, R.G.G, Beard, D.J., Cameron, E.C, Douglas, D.L., Forrest, A.R.W., Guilland-Cumming, D., Paterson, A.D., Poser, J., Preston, C.J., Milford-Ward, A., Woodhead, S. and Kanis, J.A., Biochemical markers of bone turnover in Paget's disease. Metab. Bone Dis. Rel. Res. 1981; 4 and 5: 255–262.
19. Uebelhart, D., Gineyts, E.C., Chapuy, M.C. and Delmas, P.D., Urinary excretion of pyridinium crosslinks: a new marker of bone resorption in metabolic bone disease. Bone Miner. 1990; 8: 87–96.
20. Hamdy, N.A.T., Papapoulos, S.E., Colwell, A., Eastell, R. and Russell, R.G.G., Urinary collagen crosslink excretion: a better index of bone resorption than hydroxyproline in Paget's disease of bone? Bone Miner. 1993; 22: 1–8.
21. Simon, L.S., Krane, S.M., Wortman, P.D., Krane, I.M. and Kovitz, K.L., Serum of Type I and III procollagen fragments in Paget's disease of bone. J. Clin. Endocrinol. Metab. 1984; 58: 110–120.
22. Papapoulos, S.E., Frolich, M., Mudde, A.H., Harinck, H.I.J., VDBerg, H. and Bijvoet, O.L.M., Serum osteocalcin in Paget's disease of bone: basal concentrations and response to bisphosphonate treatment. J. Clin. Endocrinol. Metab. 1987; 65: 89–94.
23. McCloskey, E.V., Yates, A.J.P., Gray, R.E.S., Hamdy, N.A.T, Galloway, J. and Kanis, J.A., Diphosphonates and phosphate homeostasis. Clin. Sci. 1988; 74: 607–612.
24. Stevens, J., Orthopaedic aspects of Paget's disease. Metab. Bone Dis. Rel. Res. 1981; 3: 271–278.
25. Herzberg, L. and Bayliss, E, Spinal cord syndrome due to non-compressive Paget's disease of bone. A spinal artery steal phenomenon reversible with calcitonin. Lancet 1980; 2: 13–15.
26. Smith, J., Botet, J.F and Yeh, S.D.J., Bone sarcomas in Paget's disease: a study of 85 patients. Radiology 1984: 152: 583–590.
27. Wick, M.R., Siegal, G.P., Unni, K.K., McLeod, R.A. and Greditzer, H.B., Sarcomas of bone complicating osteitis deformans ('Paget's disease'). Am. J. Surg. Pathol. 1981; 5: 47–59.
28. Howarth, S. Cardiac output in osteitis deformans. Clin. Sci. 1953; 12: 271–275.

Guidelines on the Management of Paget's Disease of Bone*

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Introduction

Paget's disease is a chronic focal abnormality of bone turnover. Although there remains considerable controversy regarding its etiology, several different methods of treatment have now become available. In this review, a working party derived from members of the Bone and Tooth Society and the National Association for the Relief of Paget's Disease, has examined the evidence currently available regarding the diagnosis and treatment of Paget's disease in order to develop guidelines to assist in the management of this condition.

In assessing the evidence available we have adopted the guidelines prepared by the U.S. Agency for Health Care Policy and Research[128]), including:

Ia. Evidence from meta-analyses of randomized controlled trials (RCTs).
Ib. Evidence from at least one RCT.
IIa. Evidence from a controlled study without randomization.
IIb. Evidence from another type of well-designed experimental study.
III. Evidence from well-designed nonexperimental descriptive studies (these would include comparative studies, cohort studies, and case-control studies).
IV. Expert opinions or clinical experience.

The recommendations that we made on the basis of this evidence have been graded according to the level of the evidence that supports them. These were:

A. Based on evidence level I.
B. Based on evidence levels II or III.
C. Based on evidence level IV.

Background

Paget's disease is a disorder in which there is a marked increase in bone turnover in localized parts of the skeleton. This results in an abnormal bone leading to expansion, structural weakness resulting in deformity and an increased risk of fracture, and pain. The alterations in bone shape result in mechanical changes but also can lead to pressure effects causing pain in adjacent joints

and nerve compression syndromes. Perhaps the most important nerve compression problem is involvement of the skull base leading to deafness. The abnormal bone has increased metabolic activity and blood flow, which in itself contributes to the pain and can also increase neurological complications as part of a vascular steal syndrome.

Epidemiology

Paget's disease appears to be particularly prevalent in populations of northern European ancestry. A radiological study in the UK undertaken in the 1970s suggested that the prevalence at that time might be of the order of 5.4% of the population over the age of 55 years (grade III[19]). There is a marked age dependency such that the prevalence in patients over the age of 85 years was nearly five times greater than that seen in those under aged 60 years. A similar survey undertaken at the same time in the USA suggested that the prevalence there was lower, at 2.3% of the population between the ages of 65 and 74 years (grade III[9]). Like the British study, this survey also demonstrated a marked age dependency. A more recent study conducted using the same methodology and in some of the same towns within the UK suggested that the prevalence of Paget's disease has decreased over the intervening 20 years (grade III[35]). The estimate for the prevalence of Paget's disease in patients over the age of 55 has decreased to 2%, but the increasing incidence with age was maintained. Based on this study, the investigators estimated that 118,000 women and 144,000 men in England and Wales have Paget's disease.

There is no information regarding the changes in prevalence of Paget's disease within North America, but a study from New Zealand suggested a similar decline in prevalence in that country. In this study, clinical data from 1041 patients attending a Paget's disease clinic were studied. Over the period from 1973 to 1993, there was a reduction in the proportion of patients with severe disease as judged by plasma alkaline phosphatase activity and severity of involvement on isotope bone scan (level III[36]).

Clinical Burden

Although it is generally accepted that most patients with Paget's disease are asymptomatic, there is no robust evidence of the prevalence of symptoms in patients with radiological Paget's disease. It is commonly accepted that around 5% of patients have symptoms (level IV[72]), but estimates vary considerably. Accordingly, it is difficult to assess the true burden of Paget's disease in the general population. A questionnaire survey of the Paget

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Table 1. Clinical features of Paget's disease (from refs. [65] and [75], level IV)

- Pain
  Bone pain
  Joint pain
- Deformity
  Bowing of long bones
  Skull deformities
- Fracture
  Complete
  Fissure fracture
- Neurological
  Deafness
  Other cranial nerve palsies
  Spinal cord compression
- Neoplastic transformation

Foundation in the USA reported depression in nearly half the responders, with less 25% reporting that their health was very good or excellent (level III[58]). However, a follow-up study indicated that much of the perceived ill-health was related to age and comorbid conditions, although the patient's perception of Paget's disease impact was an important determinant of health status (level III[59]). There is no reliable information available regarding the costs of Paget's disease and its treatment.

Diagnosis

Clinical

Paget's disease may present with obvious signs or symptoms or be an incidental finding during investigation of other conditions. Typical clinical features are listed in **Table 1.**

Radiological

Plain radiographs. The diagnosis of Paget's disease is primarily radiological. A number of different radiological features have been described by a variety of investigators (Table 2, level III[79,124,136]). Although a large number of differential diagnoses must be considered,[73] radiological diagnosis is usually not a problem. Where plain radiology is equivocal, computed tomography may be helpful, particularly if a high-resolution technique is used to demonstrate internal skeletal structure.

Plain radiographs are less sensitive than scintigraphy in the diagnosis of Paget's disease (see later). Therefore, there is no benefit to be obtained from using a skeletal survey of plain radiographs to assess the extent of skeletal involvement when isotope scanning would be more sensitive and involve less radiation.

Plain radiographs also are valuable in the diagnosis of secondary complications of Paget's disease such as arthritis or fracture.

- We recommend that the diagnosis of Paget's disease be confirmed with plain radiology of at least one skeletal site in all patients with the condition (grade C).
- Full skeletal survey is not usually appropriate to establish the extent of skeletal involvement.
- Any painful areas in Paget's disease should be examined by plain radiographs to determine whether there is an underlying cause.

Scintigraphy. Isotope bone scanning using ^{99m}Tc-labeled bisphosphonate tracer is more sensitive than plain radiography in

the identification of pagetic lesions. One study has suggested that such isotope scanning will detect up to 50% more lesions than are visible on plain films (level III[135]). The technique also has the advantage of being able to visualize the whole skeleton and thus assess the extent of disease.

As knowledge of disease distribution is useful in planning treatment (especially to determine whether or not the base of the skull is involved) isotope scintigraphy should be considered in all patients at presentation to assess disease extent and activity.

In contrast, scintigraphy is less specific than plain radiography and changes detected by isotope bone scanning may need to be confirmed by conventional radiography of at least one site.

Conventional isotope scintigraphy delivers a radiation dose of 3–5 mSv. Although this is equivalent to the background radiation received over a period of 2–3 years, it is comparable to the dose delivered by other tests that may be used in patients with Paget's disease. Thus, a pelvic radiograph is associated with a radiation exposure of 0.7–1.4 mSv, and lumbar spine films deliver 1.3–2.7 mSv. The dose is less than with some other measurements; for instance, CT scanning of the trunk can deliver from 5 to 15 mSv.

Attempts have been made to quantify the results of scintigraphy, but these are best considered research techniques and do not have an accepted place in clinical practice.

- We recommend that, although isotope scintigraphy is not the method of choice for the diagnosis of Paget's disease, all patients with Paget's disease should have scintigraphy performed to assess the extent of skeletal involvement (grade C).

Biochemical

Paget's disease is associated with increased bone turnover. It is therefore expected that markers of bone turnover will be increased in active disease. Total plasma alkaline phosphatase activity is elevated in 85% of patients with untreated Paget's disease (level IV[45]). In many of the patients with "normal" alkaline phosphatase activity the disease is monostotic or confined to a small number of bones (level IV[38]). There is a strong relationship between the extent of disease activity measured by scintigraphy and the degree of the elevation of alkaline phosphatase in untreated Paget's disease (level IIb[74,89]). In patients with monostotic disease, bone specific alkaline phosphatase may still be elevated. In patients with abnormal liver function, or other causes of elevated alkaline phosphatase activity not due to bone, bone specific alkaline phosphatase might be a reasonable means of assessing disease activity.

In a comparative study of different markers of bone turnover in patients with Paget's disease, the highest diagnostic sensitivity was 84%, obtained with bone specific alkaline phosphatase. The next most sensitive marker was total alkaline phosphatase, with a sensitivity of 74% (level IIb[12]). Although Paget's disease is primarily the result of disordered osteoclastic bone resorption the markers of bone resorption performed less well with lower sensitivity (level IIb[12]).

- Bone specific alkaline phosphatase is less readily available than total alkaline phosphatase and does not exhibit major benefits over the more readily available total alkaline phosphatase. We recommend that plasma total alkaline phosphatase activity be used as the standard marker of bone turnover in patients with Paget's disease (grade B).
- In patients with Paget's disease, but without an elevation of plasma total alkaline phosphatase activity, we recommend the use of bone specific alkaline phosphatase as a marker of bone turnover (grade B).

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○ In patients with liver disease we recommend the use of bone specific alkaline phosphatase to monitor the activity of Paget's disease.

Histological

Bone biopsy is rarely required to establish the diagnosis of Paget's disease. Occasionally, it may be useful in differentiation from osteoblastic metastases or osteosarcoma (grade C).

Treatments for Paget's Disease

Symptomatic

The main symptom of Paget's disease is pain. In some cases this seems to arise in association with elevated bone turnover, but may also be due to nerve compression as the result of bone deformity or coexisting arthritis. All patients need careful clinical assessment to determine the likely cause of the pain so that appropriate treatment can be given.

Paget's bone pain resulting from increased bone turnover responds well to osteoclast inhibitors, whereas that arising from nerve compression and osteoarthritis does not. These causes of pain should be treated with standard measures, such as simple analgesics, nonsteroidal anti-inflammatory agents, or opioid analgesia, either individually or in combination. Some patients will also benefit from the addition of low-dose tricyclic antidepressant therapy to an analgesic regimen. Physical methods of pain control may also be helpful. These include acupuncture, transcutaneous electrical nerve stimulation, physiotherapy, and hydrotherapy. Some patients may also derive considerable benefit from different aids and appliances such as walking sticks and frames, and also shoe raises. Joint replacement should be considered in patients with advanced osteoarthritis whose symptoms are resistant to medical therapy. Surgery may also be required in nerve compression syndromes that have not responded to medical treatment.

Specific

Specific therapy for Paget's disease is aimed at decreasing the abnormal bone turnover. Because the primary defect appears to be in the osteoclast, most treatments are aimed at decreasing osteoclastic bone resorption.

Different therapies have been used over the years. However, since the introduction of the bisphosphonates, these have increasingly assumed the prime role in the management of Paget's disease. We therefore consider the use of these agents in some detail, but spend less time discussing the use of other agents more likely to be considered only when bisphosphonate therapy has failed. Because of this, their use is likely to be confined to specialist centers.

Comparison of different treatment regimens is made difficult by the lack of any generally agreed-upon standard for therapeutic response. Many of the earliest reports have described the response in terms of percentage fall in alkaline phosphatase activity. This perspective is seriously flawed because it does not take account of the absolute level of alkaline phosphatase in the patients, and it is not informative as to the number who obtain biochemical remission with normal alkaline phosphatase activity.

Bisphosphonates. Bisphosphonates are a class of drugs related to the naturally occurring mineralization inhibitor, pyrophosphate. They were initially developed for use in the detergent industry where they prevent the formation of lime scale within pipes. However, in biological systems their basic chemical struc-

ture causes them to become bound to the surface of hydroxyapatite crystals within bone, especially on those surfaces undergoing active osteoclastic resorption.

The bisphosphonates work by two main mechanisms of action, depending on the chemical nature of the side-chain attached to the basic bisphosphonate core. Nitrogen-containing bisphosphonates, such as pamidronate, alendronate, and risedronate, inhibit enzymes of the mevalonate pathway. Among other actions, this pathway is responsible for attaching lipid moieties to small GTP-binding proteins present in the osteoclast, which are essential for cell survival and activity. Inhibition of this pathway by bisphosphonates inhibits resorptive function and triggers programmed cell death (apoptosis). Non-nitrogen-containing bisphosphonates, such as etidronate, clodronate, and tiludronate, become incorporated within stable ATP analogs, which interfere with cellular metabolic pathways, and again trigger cell death by apoptosis.

Four bisphosphonates are licensed in the UK for use in Paget's disease. These include etidronate, pamidronate, tiludronate, and risedronate. In addition, several other bisphosphonates are available in the UK for other indications and have been shown to be of use in the management of Paget's disease. These include clodronate, alendronate, and ibandronate. Other bisphosphonates, including olpadronate and zoledronate, are currently under investigation for use in Paget's disease.

All bisphosphonates have poor absorption from the gastrointestinal tract. This is compounded by the fact that they will also combine with any calcium in the stomach, further inhibiting absorption. Thus, if a bisphosphonate is given orally, it is imperative that it is not given together with food or drink containing calcium. Each bisphosphonate available on the market has different instructions for use that require adherence to ensure proper absorption.

Etidronate. Etidronate was the first bisphosphonate used in the management of Paget's disease. When given orally in doses of between 5 and 20 mg/kg per day, there was improvement in the biochemical indices of Paget's disease, as indicated by a reduction in alkaline phosphatase activity of between 40% and 70% and a similar reduction in urinary hydroxyproline excretion (level Ib[10,30,108]). Pagetic pain was also improved. Although biochemical control was better with higher doses of etidronate, these doses were associated with more adverse effects including: increased gastrointestinal side-effects (level III[77]) and increased rates of fracture (level III[30]). This latter phenomenon has subsequently been shown to be due to focal osteomalacia (level III[24]). This can occur within 2 weeks after the institution of high-dose etidronate therapy (20 mg/kg per day) for Paget's disease (level III[57]).

Use of lower doses of etidronate has been associated with lower rates of gastrointestinal side-effects (level III[77]) and fracture, similar to that seen in the general population (level III[76]). Although such regimens may be of clinical benefit (level III[8,77,120]), they have been associated with higher rates of biochemical treatment failure than the high-dose regimen. Treatment with low-dose etidronate has also been associated with long-term resistance to treatment (level III[11]).

To avoid mineralization defects, it is now recommended that etidronate be given in a dose of 400 mg/day for no longer than 6 months. After a treatment-free period of 6 months it is possible to repeat this course of therapy.

Pamidronate. Pamidronate was originally given orally in the management of Paget's disease (level II[40,52,87,126]). However, the high incidence of gastrointestinal side-effects led to its universal use as an intravenous infusion. A variety of different therapeutic regimens have been investigated (level

II[3,31,37,49,110,125,127,129,133,138]), but the currently recommended protocol is to give either three infusions of 60 mg at intervals of 2 weeks or six infusions of 30 mg over a similar time interval.

These treatments are associated with a reduction of between 50% and 80% in plasma alkaline phosphatase activity and an improvement in radiological (level III[41,49]) and scintigraphic (level III[13,97,109,131]) appearance.

Following pamidronate therapy there is often a prolonged suppression of bone turnover (level III[31,41,66,110,126,129,133,138]). This appears to be dependent on the administered dose of pamidronate and may reflect the extent to which the treatment has suppressed disease activity (level III[63,116]).

In addition to improvement in bone turnover, pamidronate therapy has also been associated with a reduction in bone pain (level II/III[13,31,52,86,87,126,138]). There are a few case reports of patients with neurological complications of Paget's disease improving following pamidronate therapy (level III[44,98,132]).

Although pamidronate is generally well tolerated, it has been associated with a substantial number of febrile reactions following intravenous therapy (level III[1,31,49]). These appear to be most common after the first infusion. In addition, some patients have experienced an increase in bone pain following pamidronate infusion (level III[31]). More serious adverse reactions are rare, but include uveitis (level III[56,85,94]), which is usually self-limiting.

The early reports of pamidronate use in management of Paget's disease found no evidence of abnormal bone mineralization (level III[52]). However, some patients receiving high-dose pamidronate treatment for Paget's disease have evidence suggestive of a mineralization defect on bone biopsy (level III[6]). The clinical significance of this is uncertain, and some have suggested that the changes were not attributable to inhibition of mineralization but merely a predictable response to the rapid changes in bone turnover rate (level IV[14]).

Tiludronate. Tiludronate is a sulfur-containing bisphosphonate that has been available for the management of Paget's disease for some years. It is normally given as a 3 month course of 400 mg as a single daily oral dose.

Early studies with tiludronate have demonstrated a 60% reduction in bone turnover (level II[16,103]). Subsequently, double-blind controlled studies have demonstrated that bone turnover markers are better suppressed by tiludronate than placebo (level Ib[51,102]) or etidronate (level III[106]). These studies suggested that tiludronate treatment is associated with a 40%–72% reduction in alkaline phosphatase activity. There was also an improvement in patients' symptoms of pagetic bone pain.

Tiludronate is usually well tolerated but is sometimes associated with looseness of the stools.

Risedronate. Risedronate is the most recent bisphosphonate to be introduced for the management of Paget's disease in the UK. Although it is a nitrogen-containing bisphosphonate, the nitrogen atom is part of a pyridinyl ring. Animal studies have suggested that risedronate may have up to 1000 times the ability of etidronate to inhibit bone resorption. In the management of Paget's disease, it is given as a single daily dose of 30 mg for a period of 2 months.

An initial uncontrolled study of 162 patients with Paget's disease indicated that the aforementioned dose of risedronate was associated with a 60%–70% reduction in alkaline phosphatase activity and improvement in bone pain in a significant number of patients (level II[121]). These results were dependent on the dose of risedronate applied (level II[27]).

A subsequent randomized, double-blind comparison of risedronate with etidronate showed that alkaline phosphatase levels were normalized in nearly 75% of patients receiving risedronate, whereas only about one in seven patients receiving etidronate achieved normal alkaline phosphatase activity (level

Ib[90]).

The improvement in bone turnover is associated with an improvement in radiological changes of the disease (level III[26]).

In the clinical trials to date, risedronate has been well tolerated without significant adverse reaction.

Clodronate. Clodronate is a first generation bisphosphonate, which has been licensed in the UK for use in malignant hypercalcemia. It has been used for the management of Paget's disease in a variety of different clinical trials but is not licensed for this indication in the UK. It is about ten times as potent as etidronate at inhibiting bone resorption but avoids the risk of inhibition of mineralization. In Paget's disease, when given either orally or intravenously, it is capable of reducing bone turnover and improving pagetic symptoms (level III[43,63,139]).

Alendronate. Alendronate is a third generation bisphosphonate that has been licensed in the UK for use in osteoporosis. In other countries it also has a license for use in the management of Paget's disease. In the latter indication the usual dose is 40 mg/day for 6 months. The 40 mg tablet is not available in the UK.

In Paget's disease, when given by either intravenous infusion or orally, alendronate has been associated with a marked reduction in bone turnover, accompanied by an improvement in bone pain (level Ib[104] and level II[2,4,5,50,80,93]). A single comparative study showed alendronate to be more potent at suppressing Paget's disease activity than etidronate (level Ib[118]). In addition, examination of radiographs showed that alendronate treatment leads to a cessation of radiological progression of Paget's disease (level III[93]) and healing of radiological lesions (level Ib[104]).

Ibandronate. Ibandronate is a potent new bisphosphonate. It is not currently available in the UK. Preliminary studies have shown that a single injection of 2 mg ibandronate is capable of suppressing disease activity in patients with Paget's disease. In patients in whom this had been insufficient to suppress disease activity, application of a higher dose was sometimes more effective (level II[62]).

Olpadronate. Olpadronate is chemically similar to pamidronate with the nitrogen atom being converted to a tertiary amine by the addition of two methyl groups. Preliminary studies in Europe and South America have suggested that the compound may be beneficial in the management of Paget's disease, but larger scale studies are awaited (level III[60,96,115,130]).

Zoledronate. Zoledronate is a potent new bisphosphonate presently under development. Preliminary studies have suggested that it may be a potent agent for the treatment of Paget's disease (level II[15,54] and level Ib[28]). It is not yet available for routine clinical use.

Comparison between bisphosphonates. Three double-blind studies have been performed in which bisphosphonates were compared with one another: Roux's study of tiludronate vs. etidronate (level Ib[106]); Siris's study of alendronate vs. etidronate (level Ib[118]); and Miller's study of risedronate vs. etidronate (level Ib[90]). In each study, etidronate was less effective than the other bisphosphonate in suppressing biochemical markers of disease activity. None of these studies showed a significant difference between the bisphosphonates in response of bone pain, but trends were observed in favor of the more potent bisphosphonate (level Ib[90,118]).

• The primary treatment for Paget's disease is inhibition of bone turnover using bisphosphonate. Oral tiludronate (400 mg/day for 12 weeks), oral risedronate (30 mg/day for 2 months), or intravenous pamidronate (three infusions of 60 mg at fortnightly intervals or six infusions of 30 mg at weekly intervals) have all been shown to be effective (grade A).

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- As other oral bisphosphonates have greater activity and fewer adverse effects, etidronate is not recommended in the management of Paget's disease (grade B).

Calcitonins. Calcitonin is a 32-amino-acid peptide secreted by the C cells of the thyroid. Its primary physiological action appears to be the suppression of plasma calcium concentration by a combination of reduced bone resorption and increased urinary calcium losses. Its physiological significance in land-living mammals is unclear, although it is clearly of major homeostatic importance in fish. It inhibits bone resorption by a direct action on osteoclasts, which is mediated by calcitonin receptors that are found on those cells. Prior to the introduction of bisphosphonates, calcitonin was the treatment of choice for the management of Paget's disease. Studies have shown that it is capable of inhibiting the activity of pagetic bone (level II[20,71,117]), reducing the symptoms of Paget's disease (level II[20,71,117]), and improving the radiological appearance of Paget's lesions (level II[82]).

As a polypeptide, calcitonin is rapidly degraded in the gastrointestinal tract and therefore needs to be given parenterally. Initially, this was done using either subcutaneous or intramuscular injections. Both these routes of administration have been associated with significant side-effects, including flushing and nausea and vomiting (level II[55,82]). More recently, it has been possible to administer calcitonin via a nasal spray with similar benefit, but with fewer side-effects (level II[39,99,137]).

In view of the weaker activity, shorter duration of action, and adverse side-effect profile compared with bisphosphonates, we do not recommend the use of calcitonin for the first line management of Paget's disease. It may have a role in those patients in whom bisphosphonates are not tolerated or have proven to be ineffective (grade B).

Plicamycin. Plicamycin (formerly mithramycin) is a cytotoxic antibiotic capable of inhibiting osteoclast activity. Although it is capable of reducing bone turnover and bone pain in patients with Paget's disease (level III[7,47,48,67,107,111,112]), these effects are limited by severe systemic toxicity. In particular, there are problems with both marrow and hepatic toxicity. The drug is no longer routinely available in the UK and we cannot recommend its use outside specialist centers and, even then, only in extreme circumstances (grade B).

Other agents. A variety of other agents have been used for the management of Paget's disease. These include gallium nitrate, glucagon, corticosteroids, and a variety of cytotoxic agents. None of these can be recommended in the routine management of Paget's disease (grade B).

Surgery

Within the management of Paget's disease, surgery is generally confined to the management of fracture, deformity, or arthritis. Although it has been suggested that, because pagetic bone is more vascular, there is increased risk of blood loss during surgery (level III[61]), this has not been reported by all investigators (level III[84,122]). Nonetheless, it would appear reasonable to administer antipagetic therapy before surgery, if only to make sure that treatment of the underlying disease has not removed the need for surgery.

Fracture. An increased rate of malunion has been reported for fractures through pagetic bone. This appears to be particularly the case for proximal femur fractures (level III[22,25]). The investigators recommend medical treatment for such patients prior to surgery (level IV), although, given the mode of presentation of such fractures, an approach of this type is often impractical. At other sites, conventional surgical techniques appear to be successful.

Deformity. Osteotomy has been used to correct deformity of Paget's disease, particularly if it causes pain or is associated with fissure fractures. More recently, some of the newer surgical techniques using Ilizarov fixators have been reported to be successful in this situation (level III[83]). No randomized studies of surgical correction of pagetic deformity have been undertaken.

- Patients with painful pagetic deformities, particularly if these are associated with fissure fracture, should be considered for surgical treatment (grade B).

Arthritis. There is considerable experience with regard to arthroplasty for the management of osteoarthritis in patients with Paget's disease. Most recent series have reported results similar to those achieved in nonpagetic patients, although there is a slight increase in the risk of heterotopic ossification and nonunion of the trochanter in hip arthroplasty (level III[84,122]).

- Patients with osteoarthritis related to Paget's disease, whose symptoms do not settle with medical therapy, should be considered for surgery (grade B).

Indications for Treatment of Paget's Disease

A number of different criteria for the treatment of Paget's disease have been put forward (level IV[38,69,119]). We examine the evidence for each of these indications in turn.

Bone Pain

Bone pain is the only complication of Paget's disease for which there is firm evidence that specific antipagetic therapy is associated with clinical benefit. There have been five placebo-controlled, double-blind studies of bisphosphonate treatment in Paget's disease in which pain was assessed as one of the end-points, and four of these showed that bisphosphonate was superior to placebo (level I[10,102,104,78,101]). Other comparative studies of bisphosphonates have shown that pain relief tends to be better with more powerful bisphosphonates, although the differences between treatments were not statistically significant (level I[90,106,118]).

Pain relief has also been reported with calcitonin (level II[34,71]) and plicamycin (level II[67,113]), but this has not been demonstrated within the context of a randomized, controlled clinical trial.

- Pain in pagetic bone is a definite indication for antipagetic treatment (grade A).

Fracture

Fracture is a common complication of Paget's disease, but the effects of antipagetic therapy on fracture have not been systematically studied. Indeed, one early study of etidronate suggested that fracture risk was increased with high-dose treatment[30]; this was probably the result of impaired mineralization.

It might be expected that agents that reduce bone turnover in Paget's disease would improve fracture risk. However, only two placebo-controlled studies with detailed information on fractures have been performed. One study found that 1 of 9 placebo-treated patients suffered a fracture during a 6 month follow-up period compared with 2 of 41 etidronate-treated patients (level I[10]). In another short-term study with oral tiludronate, one fracture occurred in the 400 mg dose group and none in the placebo group (level I[51]). These differences were not significant in either study. However, both these studies had very low power to detect change in fracture risk, and thus it would be inappro-

priate to conclude that bisphosphonates do not reduce fracture risk. Further research is indicated.

The effect of medical treatment on fissure fractures has not been studied.

There is no evidence that treatment of Paget's disease improves healing of fractures.

- Treatment of Paget's disease solely to reduce fracture risk is not indicated (grade C).
- Treatment of Paget's disease following fracture to improve healing is not justified. (grade C).

Prevention of Bone Deformity

Bone deformity, particularly affecting weight-bearing long bones, is a common complication of Paget's disease. Suppression of bone turnover might be expected to help prevent bone deformity on a theoretical basis, but the effects of antipagetic therapy on bone deformity have not been assessed in controlled clinical trials.

Clinical observations in a small number of patients have indicated that bisphosphonate therapy might reduce facial deformities in patients with Paget's disease (level III[21,29]).

- The effects of antipagetic therapy on bone deformity are unclear (grade C).
- Bisphosphonate therapy may be justified in the management of facial deformities due to Paget's disease (grade B).
- Therapy of Paget's disease cannot be justified solely for the prevention of deformity elsewhere (grade C).

Osteolytic Lesions

There is evidence from a controlled trial to suggest that alendronate promotes radiological healing of osteolytic lesions in Paget's disease (level I[104]). Uncontrolled studies have shown healing of osteolytic lesions with other bisphosphonates (level II[26,41,49]), suggesting that this may be a class effect of all potent bisphosphonates. The clinical significance of this is unclear and we do not make specific recommendations about the treatment of osteolytic disease in the absence of other indications for treatment.

Prevention of Osteoarthritis

The risk of osteoarthritis is increased in Paget's disease, but there is no evidence that antipagetic therapy affects the development or progression of osteoarthritis:

- Because there is often diagnostic difficulty in distinguishing between pagetic bone pain and the pain of osteoarthritis in an adjacent joint, it is not unreasonable to treat patients in whom there is uncertainty as to the cause of pain (grade C).

Deafness

Deafness is a common complication of Paget's disease, but the effects of antipagetic treatment on development or progression of deafness are poorly understood. No controlled studies have been undertaken; however, several small clinical series have suggested that treatment of Paget's disease (mainly with calcitonin) can reduce the rate of progression of hearing loss, although no improvement in hearing has been seen (level III[46,81,88,123]). In view of this, and the irreversibility of hearing loss if it does occur, many investigators have recommended treating Paget's disease of the skull base to minimize the risk of developing deafness.

- In patients with Paget's disease of the skull base, treatment should be considered to minimize the risk of hearing loss (grade B).

Quality of Life

Two studies have examined the short-term effects of bisphosphonates on functional status in Paget's. Both of these studies examined the effects of bisphosphonate therapies and neither demonstrated any significant difference following treatment (level Ib[90,118]). It must be remembered that quality of life was not the primary end-point in either of these studies and that neither study was designed with the appropriate power to detect small changes in quality of life.

Spinal Cord Compression

Several case reports have demonstrated the ability of calcitonin (level III[33,43,64,68,114,134]) and bisphosphonates (level III[32,43,98,132]) to improve neurological function in patients with Paget's disease and spinal cord dysfunction. This is in contrast to surgery, which has been associated with problems in a significant number of patients (level III[44] and level IV[76]). As these are relatively rare complications, it is unlikely that it will be possible to undertake a randomized comparison of surgery and medical intervention.

In such patients it is important to consider causes of spinal compression other than Paget's disease.

- Patients who develop neurological symptoms as a result of spinal Paget's disease should initially be treated medically. If this fails to relieve symptoms then surgical decompression should be considered (grade B).

Blood Loss

It has also been suggested that antipagetic therapy given preoperatively may reduce intraoperative blood loss (level IV[23]), although this has been disputed by others (level III[84]). The effect of antipagetic therapy on operative blood loss has never been studied in a randomized trial.

Hypercalcemia

Hypercalcaemia is a rare complication of Paget's disease due to a combination of increased bone turnover and either immobilization (level III[18,53,91]) or hyperparathyroidism (level III[95,100]). Clinical observations have suggested that treatment of the underlying Paget's disease might be helpful in these circumstances (level III[17]). Given the benefit of bisphosphonates in hypercalcaemia due to other causes it is reasonable to offer such treatment to hypercalcemic patients with Paget's disease.

- Patients with Paget's disease and hypercalcemia should be treated with bisphosphonate (grade B).

Sarcoma

There is no evidence that treatment affects either the development or progression of Paget's sarcomata.

Young Age

Some experts have advised that patients presenting with Paget's disease at a young age should be given treatment regardless of other indications. There is no evidence to support this contention.

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Monitoring Treatment

The ultimate aim of treatment is to relieve the symptoms and prevent the complications of Paget's disease. In general terms, this is only measurable in a practical way for pain and radiological improvement of osteolytic lesions. In certain very specific situations, outlined in what follows, other clinical changes can be useful.

Improvement in the biochemical markers of excess bone turnover is easily measured and provides the most rapid indication of treatment effect. Although the optimum levels of reduction in bone turnover are not fully established, the consensus view is that biochemical markers ideally should be suppressed into the population reference (normal) range.

Biochemical Response to Treatment

Total serum alkaline phosphatase activity is the most commonly used biochemical marker of disease activity (see earlier). It has good reproducibility (coefficient of variation 10%) and is sensitive to clinically important changes in disease activity. It has generally been accepted that a fall of 25% in total alkaline phosphatase activity represents a significant treatment response (grade C).

Bone specific alkaline phosphatase is more sensitive and specific that total alkaline phosphatase (level IIb[12]), although this difference is unlikely to be of clinical importance for the majority of patients. The situations where this benefit may be of importance include patients with liver disease, patients with monostotic disease, and those with total alkaline phosphatase within the normal range. In these patients, consideration should be given to using bone specific alkaline phosphatase to monitor disease progress.

In all studies of bisphosphonate therapy the nadir alkaline phosphatase level occurred 3–6 months after commencement of therapy, followed by a gradual offset of treatment effect (level Ib). We therefore recommend that bone turnover be measured every 3 months for the first 6 months of therapy, and thereafter at intervals of 6 months (grade C).

Urinary markers of bone resorption such as deoxypyridinoline or hydroxyproline respond more quickly to treatment (nadir within 1 month posttreatment) and may also indicate relapse before changes in alkaline phosphatase occur. The reproducibility of these markers is not as good, however, and 50% changes are required at the individual level to be significant. There is therefore little clinical benefit to be obtained from the routine clinical use of resorption markers.

Isotope Bone Scans

Isotype bone scans are a rather insensitive method of response measurement and there is considerable delay between biochemical response and improvement in bone scan (about 6 months). It also exposes patients to extra doses of radiation. However, in monostotic disease and situations wherein there is persistent pain despite normal bone biochemistry, repeat scans may be useful (grade C).

When Should Patients Be Retreated?

If patients do relapse they can be re-treated effectively, especially with potent bisphosphonates (level II[105,121]).

There have been no clinical trials specifically evaluating re-treatment of Paget's disease following relapse, but a consensus exists that re-treatment is indicated when there is (all grade C):

Table 2. Radiological features of Paget's disease

- Early disease—primarily lytic
  V-shaped "cutting cone" in long bones
  Osteoporosis circumscripta in skull
- Combined phase (mixed lytic and sclerotic)
  Cortical thickening
  Loss of corticomedullary distinction
  Accentuated trabecular markings
- Late phase—primarily sclerotic
  Thickening of long bones
  Increase in bone size
  Sclerosis

1. *Symptom relapse or persistence* This is particularly the case for pain but should be confirmed by objective evidence of continuing disease activity. In the absence of evidence of continuing disease activity other causes of pain should be sought.
2. *Biochemical relapse* In those instances where treatment has been based on the presence of asymptomatic disease in a critical site, it is necessary to base re-treatment on biochemical criteria. There is no clinical trial evidence on which to base criteria for this. However, it is generally accepted that an increase of alkaline phosphatase of 25% above nadir (even if the total is still within the normal range) indicates significant relapse.

As the effects of treatment are generally apparent by 3 months after introduction of therapy, and maximal by 6 months, it is appropriate to offer re-treatment if a patient has failed to respond 6 months after treatment. There is some anecdotal experience to suggest that such patients may respond to administration of a bisphosphonate that is more potent than that given originally (grade C). (Table 2; [42,92])

References

1. Adami, S., Bhalla, A. K., Dorizzi, R., Montesanti, F., Rosini, S., Salvagno, G., et al. The acute-phase response after bisphosphonate administration. Calcif Tissue Int 41:326–331; 1987.
2. Adami, S., Mian, M., Gatti, P., Rossini, M., Zamberlan, N., Bertoldo, F., et al. Effects of two oral doses of alendronate in the treatment of Paget's disease of bone. Bone 15:415–417; 1994.
3. Adami, S., Salvagno, G., Guarrera, G., Montesanti, F., Garavelli, S., Rosini, S., et al. Treatment of Paget's disease of bone with intravenous 4-amino-1-hydroxybutylidene-1,1-bisphosphonate. Calcif Tissue Int 39:226–229; 1986.
4. Adami, S., Zamberlan, N., Mian, M., Dorizzi, R., Rossini, M., Braga, B., et al. Duration of the effects of intravenous alendronate in postmenopausal women and in patients with primary hyperparathyroidism and Paget's disease of bone. Bone Miner 25:75–82; 1994.
5. Adami, S., Zamberlan, N., Mian, M., Dorizzi, R., Rossini, M., Braga, B., et al. Duration of the effects of intravenous alendronate in postmenopausal women and in patients with primary hyperparathyroidism and Paget's disease of bone. Bone Miner 25:75–82; 1994.
6. Adamson, B. B., Gallacher, S. J., Byars, J., Ralston, S. H., Boyle, I. T., and Boyce, B. F. Mineralisation defects with pamidronate therapy for Paget's disease. Lancet 342:1459–1460; 1993.

7. Aitken, J. M. and Lindsay, R. Mithramycin in Paget's disease. Lancet i:1177–1178; 1973.

8. Alexandre, C., Meunier, P. J., Edouard, C., Khairi, R. A., and Johnston, C. C. Effects of ethane-1 hydroxy-1,1-diphosphonate (5 mg/kg/day dose) on quantitative bone histology in Paget's disease of bone. Metab Bone Dis Rel Res 3:309–315; 1981.

9. Altman, R. D., Bloch, D. A., Hochberg, M. C., and Murphy, W. A. Prevalence of pelvic Paget's disease of bone in the United States. J Bone Miner Res 15:461–465; 2000.

10. Altman, R. D., Johnston, C. C., Khairi, M. R., Wellman, H., Serafini, A. N., and Sankey, R. R. Influence of disodium etidronate on clinical and laboratory manifestations of Paget's disease of bone (osteitis deformans). N Engl J Med 289:1379–1384; 1973.

11. Altman, R. D. Long-term follow-up of therapy with intermittent etidronate disodium in Paget's disease of bone. Am J Med 79:583–590; 1985.

12. Alvarez, L., Guanabens, N., Peris, P., Monegal, A., Bedini, J. L., Deulofeu, R., et al. Discriminative value of biochemical markers of bone turnover in assessing the activity of Paget's disease. J Bone Miner Res 10:458–465; 1995.

13. Anderson, D. C., O'Driscoll, J. B., Buckler, H. M., Cantrill, J., and Brown, J. D. Relapse of osteoporosis circumscripta as a lytic ring after treatment of Paget's disease with intravenous 3-amino-1-hydroxypropylidene-1,1-bisphosphonate. Br J Radiol 61:996–1001; 1988.

14. Anderson, D. C. and Richardson, P. C. Pamidronate and Paget's disease [letter]. Lancet 343:427; 1994.

15. Arden-Cordone, M., Siris, E. S., Lyles, K. W., Knieriem, A., Newton, R. A., Schaffer, V., et al. Antiresorptive effect of a single infusion of microgram quantities of zoledronate in Paget's disease of bone. Calcif Tissue Int 60:415–418; 1997.

16. Audran, M., Clochon, P., Etghen, D., Mazieres, B., and Renier, J. C. Treatment of Paget's disease of bone with (4-chloro-phenyl) thiomethylene bisphosphonate. Clin Rheumatol 8:71–79; 1989.

17. Auld, W. H. R., Simpson, R. H., and Smyth, M. Hypercalcaemia in Paget's disease of bone. Lancet i:562–563; 1979.

18. Bannister, P., Roberts, M., and Sheridan, P. Recurrent hypercalcaemia in a young man with mono-ostotic Paget's disease. Postgrad Med J 62:481–483; 1986.

19. Barker, D. J., Clough, P. W., Guyer, P. B., and Gardner, M. J. Paget's disease of bone in 14 British towns. Br Med J 1:1181–1183; 1977.

20. Bell, N. H., Avery, S., and Johnston, C. C. Jr. Effects of calcitonin in Paget's disease and polyostotic fibrous dysplasia. J Clin Endocrinol Metab 31:283–290; 1970.

21. Bickerstaff, D. R., Douglas, D. L., Burke, P. H., O'Doherty, D. P., and Kanis, J. A. Improvement in the deformity of the face in Paget's disease treated with diphosphonates. J Bone Jt Surg [Br] 72:132–136; 1990.

22. Bidner, S. and Finnegan, M. Femoral fractures in Paget's disease. J Orthop Trauma 3:317–322; 1989.

23. Bowie, H. I. C. and Kanis, J. A. Calcitonin in the assessment and preparation of patients with Paget's disease for surgery. In: Kanis, J. A., ed. Bone Disease and Calcitonin. Eastbourne, UK: Armour Pharmaceuticals; 61–69; 1976.

24. Boyce, B. F., Smith, L., Fogelman, I., Johnston, E., Ralston, S., and Boyle, I. T. Focal osteomalacia due to low-dose diphosphonate therapy in paget's disease. Lancet i:821–824; 1984.

25. Bradley, C. M. and Nade, S. Outcome after fractures of the femur in Paget's disease. Austral NZ J Surg 62:39–44; 1992.

26. Brown, J. P., Chines, A. A., Myers, W. R., Eusebio, R. A., Ritter-Hrncirik, C., and Hayes, C. W. Improvement of pagetic bone lesions with risedronate treatment: A radiologic study. Bone 26:263–267; 2000.

27. Brown, J. P., Hosking, D. J., Ste-Marie, L., Johnston, C. C. Jr., Reginster, J., Ryan, W. G., et al. Risedronate, a highly effective, short-term oral treatment for Paget's disease: A dose-response study. Calcif Tissue Int 64:93–99; 1999.

28. Buckler, H., Fraser, W., Hosking, D., Ryan, W., Maricic, M. J., Singer, F., et al. Single infusion of zoledronate in Paget's disease of bone: A placebo-controlled, dose-ranging study. Bone 24(Suppl. 5):81S–85S; 1999.

29. Buckler, H. M., Cantrill, J. A., Klimiuk, P. S., and Anderson, D. C. Paget's disease of the orbit: Before and after APD. Br Med J Clin Res Ed 295:1655; 1987.

30. Canfield, R., Rosner, W., Skinner, J., McWhorter, J., Resnick, L., Feldman, F., et al. Diphosphonate therapy of Paget's disease of bone. J Clin Endocrinol Metab 44:96–106; 1977.

31. Cantrill, J. A., Buckler, H. M., and Anderson, D. C. Low dose intravenous 3-amino-1-hydroxypropylidene-1,1-bisphosphonate (APD) for the treatment of Paget's disease of bone. Ann Rheum Dis 45:1012–1018; 1986.

32. Charhon, S., Chapuy, M. C., Valentin-Opran, A., and Meunier, P. J. Intravenous etidronate for spinal cord dysfunction due to Paget's disease [letter]. Lancet i:391–392; 1982.

33. Chen, J. R., Rhee, R. S., Wallach, S., Avramides, A., and Flores, A. Neurologic disturbances in Paget disease of bone: Response to calcitonin. Neurology 29:448–457; 1979.

34. Condon, J. R., Surtees, J., and Robinson, V. Control of osteitis deformans using glucagon, calcitonin, and mithramycin. Postgrad Med J 57:84–88; 1981.

35. Cooper, C., Schafheutle, K., Dennison, E., Kellingray, S., Guyer, P., and Barker, D. The epidemiology of Paget's disease in Britain: Is the prevalence decreasing? J Bone Miner Res 14:192–197; 1999.

36. Cundy, T., McAnulty, K., Wattie, D., Gamble, G., Rutland, M., and Ibbertson, H. K. Evidence for secular change in Paget's disease. Bone 20:69–71; 1997.

37. Cundy, T., Wattie, D., and King, A. R. High-dose pamidronate in the management of resistant Paget's disease. Calcif Tissue Int 58:6–8; 1996.

38. Delmas, P. D. and Meunier, P. J. Drug therapy: The management of Paget's disease of bone. N Engl J Med 336:558–566; 1997.

39. Devogelaer, J. P., Azria, M., Attinger, M., Abbiati, G., Castiglioni, C., and Nagant-de-Deuxchaisnes, C. Comparison of the acute biological action of injectable salmon calcitonin and an injectable and oral calcitonin analogue. Calcif Tissue Int 55:71–73; 1994.

40. Dewis, P., Prasad, B. K., Anderson, D. C., and Willets, S. Clinical experience with the use of two diphosphonates in the treatment of Paget's disease. Ann Rheum Dis 44:34–38; 1985.

41. Dodd, G. W., Ibbertson, H. K., Fraser, T. R., Holdaway, I. M., and Wattie, D. Radiological assessment of Paget's disease of bone after treatment with the bisphosphonates EHDP and APD. Br J Radiol 60:849–860; 1987.

42. Douglas, D. L., Duckworth, T., Kanis, J. A., Jefferson, A. A., Martin, T. J., and Russell, R. G. Spinal cord dysfunction in Paget's disease of bone. Has medical treatment a vascular basis? J Bone Jt Surg [Br] 63B:495–503; 1981.

43. Douglas, D. L., Duckworth, T., Kanis, J. A., Preston, C., Beard, D. J., Smith, T. W., et al. Biochemical and clinical responses to dichloromethylene diphosphonate (Cl2MDP) in Paget's disease of bone. Arthritis Rheum 23:1185–1192; 1980.

44. Douglas, D. L., Kanis, J. A., Duckworth, T., Beard, D. J., Paterson, A. D., and Russell, R. G. Paget's disease: Improvement of spinal cord dysfunction with diphosphonates and calcitonin. Metab Bone Dis Rel Res 3:327–335; 1981.

45. Eastell, R. Biochemical markers of bone turnover in Paget's disease of bone. Bone 24(Suppl. 5):49S–50S; 1999.

46. el Sammaa, M., Linthicum, F. H. Jr., House, H. P., and House, J. W. Calcitonin as treatment for hearing loss in Paget's disease. Am J Otol 7:241–243; 1986.

47. Elias, E. G. and Evans, J. T. Mithramycin in the treatment of Paget's disease of bone. J Bone J Surg [Am] 54:1730–1736; 1972.

48. Epstein, S. Mithramycin in Paget's disease. S Afr Med J 48:1328–1330; 1974.

49. Fenton, A. J., Gutteridge, D. H., Kent, G. N., Price, R. I., Retallack, R. W., Bhagat, C. I., et al. Intravenous aminobisphosphonate in Paget's disease: Clinical, biochemical, histomorphometric and radiological responses. Clin Endocrinol 34:197–204; 1991.

50. Filipponi, P., Pedetti, M., Beghe, F., Giovagnini, B., Miam, M., and Cristallini, S. Effects of two different bisphosphonates on Paget's disease of bone: ICTP assessed. Bone 15:261–267; 1994.

51. Fraser, W. D., Stamp, T. C., Creek, R. A., Sawyer, J. P., and Picot, C. A double-blind, multicentre, placebo-controlled study of tiludronate in Paget's disease of bone. Postgrad Med J 73:496–502; 1997.

52. Frijlink, W. B., Bijvoet, O. L., te Velde, J., and Heynen, G. Treatment of Paget's disease with (3-amino-1-hydroxypropylidene)-1, 1-bisphosphonate (A.P.D.). Lancet 1:799–803; 1979.

53. Fuss, M., Bergans, A., and Corvilain, J. Hypercalcaemia due to immobilisation in Paget's disease of bone [letter]. Lancet ii:941; 1978.

54. Garnero, P., Gineyts, E., Schaffer, A. V., Seaman, J., and Delmas, P. D. Measurement of urinary excretion of nonisomerized and beta-isomerized forms of type 1 collagen breakdown products to monitor the effects of the bisphosphonate zoledronate in Paget's disease. Arthritis Rheum 41:354–360; 1998.

55. Gennari, C., Passeri, M., Chierichetti, S. M., and Piolini, M. Side-effects of synthetic salmon and human calcitonin [letter]. Lancet i:594–595; 1983.

56. Ghose, K., Waterworth, R., Trolove, P., and Highton, J. Uveitis associated with pamidronate. Austral NZ J Med 24:320; 1994.

57. Gibbs, C. J., Aaron, J. E., and Peacock, M. Osteomalacia in Paget's disease treated with short term, high dose sodium etidronate. Br Med J 292:1227–1229; 1986.

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58. Gold, D. T., Boisture, J., Shipp, K. M., Pieper, C. F., and Lyles, K. W. Paget's disease of bone and quality of life. J Bone Miner Res 11:1897–1904; 1996.

59. Gold, D. T., Burchett, B. M., Shipp, K. M., Pieper, C. F., and Lyles, K. W. Factors associated with self-rated health in patients with Paget's disease of bone. J Bone Miner Res 14(Suppl. 2):99–102; 1999.

60. Gonzalez, D. C. and Mautalen, C. A. Short-term therapy with oral olpadronate in active Paget's disease of bone. J Bone Miner Res 14:2042–2047; 1999.

61. Goutallier, D., Sterkers, Y., and Cadeua, F. Expérience de la prosthèse totale au cours de la coxopathie pagétique. Rhumatologie 36:81–82; 1984.

62. Grauer, A., Heichel, S., Knaus, J., Dosch, E., and Ziegler, R. Ibandronate treatment in Paget's disease of bone. Bone 24(Suppl. 5):87–89; 1999.

63. Gray, R. E., Yates, A. J., Preston, C. J., Smith, R., Russell, R. G., and Kanis, J. A. Duration of effect of oral diphosphonate therapy in Paget's disease of bone. Q J Med 64:755–767; 1987.

64. Gruszkiewicz, J., Doron, Y., Borovich, B., and Zaaroor, M. Spinal cord compression in Paget's disease of bone with reference to sarcomatous degeneration and calcitonin treatment. Surg Neurol 27:117–125; 1987.

65. Hamdy, R. Paget's Disease of Bone Assessment and Management. London: Praeger; 1981.

66. Harinck, H. I., Papapoulos, S. E., Blanksma, H. J., Moolenaar, A. J., Vermeij, P., and Bijvoet, O. L. Paget's disease of bone: Early and late responses to three different modes of treatment with aminohydroxypropylidene bisphosphonate (APD). Br Med J Clin Res Ed 295:1301–1305; 1987.

67. Heath, D. A. The role of mithramycin in the management of Paget's disease. Metab Bone Dis Rel Res 3:343–345; 1981.

68. Herzberg, L. and Bayliss, E. Spinal-cord syndrome due to non-compressive Paget's disease of bone: A spinal-artery steal phenomenon reversible with calcitonin. Lancet ii:13–15; 1980.

69. Hosking, D., Meunier, P. J., Ringe, J. D., Reginster, J. Y., and Gennari, C. Paget's disease: Diagnosis and management. Br Med J 312:491–494; 1996.

70. Johnston, C. C. Jr., Altman, R. D., Canfield, R. E., Finerman, G. A., Taulbee, J. D., and Ebert, M. L. Review of fracture experience during treatment of Paget's disease of bone with etidronate disodium (EHDP). Clin Orthop Rel Res 172:186–194; 1983.

71. Kanis, J. A., Fitzpatrick, K., and Strong, J. A. Treatment of Paget's disease of bone with porcine calcitonin: Clinical and metabolic responses. Q J Med 44:399–413; 1975.

72. Kanis, J. A. Pathophysiology and Treatment of Paget's Disease of Bone. 2nd Ed. London: Martin Dunitz; 10; 1998.

73. Kanis, J. A. Pathophysiology and Treatment of Paget's Disease of Bone. 2nd Ed. London: Martin Dunitz; 139–158; 1998.

74. Kanis, J. A. Pathophysiology and Treatment of Paget's Disease of Bone. 2nd Ed. London: Martin Dunitz; 99; 1998.

75. Kanis, J. A. Pathophysiology and Treatment of Paget's Disease of Bone. 2nd Ed. London: Martin Dunitz; 110–138; 1998.

76. Kaplan, F. S. Severe orthopaedic complications of Paget's disease. Bone 24(Suppl. 5):43S–46S; 1999.

77. Khairi, M. R., Altman, R. D., DeRosa, G. P., Zimmermann, J., Schenk, R. K., and Johnston, C. C. Sodium etidronate in the treatment of Paget's disease of bone. A study of long-term results. Ann Intern Med 87:656–663; 1977.

78. Khairi, M. R., Johnston, C. C. Jr., Altman, R. D., Wellman, H. N., Serafini, A. N., and Sankey, R. R. Treatment of Paget disease of bone (osteitis deformans). Results of a one-year study with sodium etidronate. JAMA 230:526–527; 1974.

79. Khairi, M. R., Robb, J. A., Wellman, H. N., and Johnston, C. C. Jr. Radiographs and scans in diagnosing symptomatic lesions of Paget's disease of bone (osteitis deformans). Geriatrics 29:49–54; 1974.

80. Khan, S. A., Vasikaran, S., McCloskey, E. V., Beneton, M. N., Rogers, S., Coulton, L., et al. Alendronate in the treatment of Paget's disease of bone. Bone 20:263–271; 1997.

81. Lando, M., Hoover, L. A., and Finerman, G. Stabilization of hearing loss in Paget's disease with calcitonin and etidronate. Arch Otolaryngol Head Neck Surg 114:891–894; 1988.

82. Lavender, J. P., Evans, I. M., Arnot, R., Bowring, S., Doyle, F. H., Joplin, G. F., et al. A comparison of radiography and radioisotope scanning in the detection of Paget's disease and in the assessment of response to human calcitonin. Br J Radiol 50:243–250; 1977.

83. Louette, L., Lammens, J., and Fabry, G. The Ilizarov external fixator for treatment of deformities in Paget's disease. Clin Orthop Rel Res 323:298–303; 1996.

84. Ludkowski, P. and Wilson-Macdonald, J. Total arthoplasty in Paget's disease of the hip. Clin Orthop Rel Res 255:160–167; 1990.

85. Macarol, V. and Fraunfelder, F. T. Pamidronate disodium and possible ocular adverse drug reactions. Am J Ophthalmol 118:220–224; 1994.

86. Mallette, L. E. Successful treatment of resistant Paget's disease of bone with pamidronate. Arch Intern Med 149:2765–2767; 1989.

87. Mautalen, C. A., Gonzalez, D., and Ghiringhelli, G. Efficacy of the bisphosphonate APD in the control of Paget's bone disease. Bone 6:429–432; 1985.

88. Menzies, M. A., Greenberg, P. B., and Joplin, G. F. Otological studies in patients with deafness due to Paget's disease before and after treatment with synthetic human calcitonin. Acta Oto-Laryngologica 79:378–383; 1975.

89. Meunier, P. J., Salson, C., Mathieu, L., Chapuy, M. C., Delmas, P., Alexandre, C., et al. Skeletal distribution and biochemical parameters of Paget's disease. Clin Orthop Rel Res 217:37–44; 1987.

90. Miller, P. D., Brown, J. P., Siris, E. S., Hoseyni, M. S., Axelrod, D. W., and Bekker, P. J. A randomized, double-blind comparison of risedronate and etidronate in the treatment of Paget's disease of bone. Paget's Risedronate/Etidronate Study Group. Am J Med 106:513–520; 1999.

91. Nathan, A. W., Ludlam, H. A., Wilson, D. W., and Dandona, P. Hypercalcaemia due to immobilization of a patient with Paget's disease of bone. Postgrad Med J 58:714–715; 1982.

92. O'Doherty, D. P., Bickerstaff, D. R., McCloskey, E. V., Eyres, K. S., and Kanis, J. A. Radiographic healing of osteolytic Paget's disease of the tibia with alendronate. J Orthop Rheumatol 4:153–157; 1991.

93. O'Doherty, D. P., Bickerstaff, D. R., McCloskey, E. V., Hamdy, N. A., Beneton, M. N., Harris, S., et al. Treatment of Paget's disease of bone with aminohydroxybutylidene bisphosphonate. J Bone Miner Res 5:483–491; 1990.

94. O'Donnell, N. P., Rao, G. P., and Aguis-Fernandez, A. Paget's disease: ocular complications of disodium pamidronate treatment. Br J Clin Pract 49:272–273; 1995.

95. Ooi, T. C., Evans, M. C., Spiro, T. P., and Ibbertson, H. K. Coexisting Paget's disease of bone and hyperparathyroidism. NZ Med J 91:134–136; 1980.

96. Papapoulos, S. E., Hoekman, K., Lowik, C. W., Vermeij, P., and Bijvoet, O. L. Application of an in vitro model and a clinical protocol in the assessment of the potency of a new bisphosphonate. J Bone Miner Res 4:775–781; 1989.

97. Patel, U., Gallacher, S. J., Boyle, I. T., and McKillop, J. H. Serial bone scans in Paget's disease: Development of new lesions, natural variation in lesion intensity and nature of changes seen after treatment. Nucl Med Commun 11:747–760; 1990.

98. Percival, R. C., Watson, M. E., Williams, J. L., and Kanis, J. A. Carcinoma of the prostate: Remission of paraparesis with inhibitors of bone resorption. Postgrad Med J 61:551–553; 1985.

99. Pontiroli, A. E., Pajetta, E., Calderara, A., Alberetto, M., Pozza, G., Manganelli, V., et al. Intranasal and intramuscular human calcitonin in female osteoporosis and in Paget's disease of bones: A pilot study. J Endocrinol Invest 14:47–51; 1991.

100. Posen, S., Clifton-Bligh, P., and Wilkinson, M. Paget's disease of bone and hyperparathyroidism: Coincidence or causal relationship? Calcif Tissue Res 26:107–109; 1978.

101. Ralston, S. H., Boyce, B. F., Cowan, R. A., Fogelman, I., Smith, M. L., Jenkins, A., et al. The effect of 1 alpha-hydroxyvitamin D3 on the mineralization defect in disodium etidronate-treated Paget's disease—a double-blind randomized clinical study. J Bone Miner Res 2:5–12; 1987.

102. Reginster, J. Y., Colson, F., Morlock, G., Combe, B., Ethgen, D., and Geusens, P. Evaluation of the efficacy and safety of oral tiludronate in Paget's disease of bone. A double-blind, multiple-dosage, placebo-controlled study. Arthritis Rheum 35:967–974; 1992.

103. Reginster, J. Y., Jeugmans-Huynen, A. M., Albert, A., Denis, D., Deroisy, R., Lecart, M. P., et al. Biological and clinical assessment of a new bisphosphonate, (chloro-4 phenyl) thiomethylene bisphosphonate, in the treatment of Paget's disease of bone. Bone 9:349–354; 1988.

104. Reid, I. R., Nicholson, G. C., Weinstein, R. S., Hosking, D. J., Cundy, T., Kotowicz, M. A., et al. Biochemical and radiologic improvement in Paget's disease of bone treated with alendronate: A randomized, placebo-controlled trial. Am J Med 101:341–348; 1996.

105. Richardson, P. C., Cantrill, J. A., and Anderson, D. C. Experience of treating 218 patients with Paget's disease of bone using intravenous 3-aminohydroxypropylidene 1-1 bisphosphonate (Pamidronate, APD). In: Cohn, D. V., Glorieux, F. H., & Martin, T. J., eds. Calcium Regulation and Bone Metabolism. New York: Elsevier; 509–514; 1990.

106. Roux, C., Gennari, C., Farrerons, J., Devogelaer, J. P., Mulder, H., Kruse, H. P., et al. Comparative prospective, double-blind, multicenter study of the

efficacy of tiludronate and etidronate in the treatment of Paget's disease of bone. Arthritis Rheum 38:851–858; 1995.

107. Russell, A. S., Chalmers, I. M., Percy, J. S., and Lentle, B. C. Long term effectiveness of low dose mithramycin for Paget's disease of bone. Arthritis Rheum 22:215–218; 1979.

108. Russell, R. G., Smith, R., Preston, C., Walton, R. J., and Woods, C. G. Diphosphonates in Paget's disease. Lancet i:894–898; 1974.

109. Ryan, P. J., Gibson, T., and Fogelman, I. Bone scintigraphy following intravenous pamidronate for Paget's disease of bone. J Nucl Med 33:1589–1593; 1992.

110. Ryan, P. J., Sherry, M., Gibson, T., and Fogelman, I. Treatment of Paget's disease by weekly infusions of 3-aminohydroxypropylidene-1,1-bisphosphonate (APD). Br J Rheumatol 31:97–101; 1992.

111. Ryan, W. G., Schwartz, T. B., and Fordham, E. W. Mithramycin and long remission of Paget's disease of bone [letter]. Ann Intern Med 92:129–130; 1980.

112. Ryan, W. G. Mithramycin in Paget's disease of bone. Lancet i:1319; 1973.

113. Ryan, W. G. Treatment of Paget's disease of bone with mithramycin. Clin Orthop Rel Res 127:106–110; 1977.

114. Schumacher, M., Levy, A., Beck, U., and Remagen, W. Paget's disease with spinal cord compression. Favourable course after decompression and calcitonin treatment in two cases. Eur Neurol 15:116–120; 1977.

115. Schweitzer, D. H., Zwinderman, A. H., Vermeij, P., Bijvoet, O. L., and Papapoulos, S. E. Improved treatment of Paget's disease with dimethylaminohydroxypropylidene bisphosphonate. J Bone Miner Res 8:175–182; 1993.

116. Selby, P. L. Pamidronate in the treatment of Paget's disease. Bone 24(Suppl. 5):57S–58S; 1999.

117. Shai, F., Baker, R. K., and Wallach, S. The clinical and metabolic effects of porcine calcitonin on Paget's disease of bone. J Clin Invest 50:1927–1940; 1971.

118. Siris, E., Weinstein, R. S., Altman, R., Conte, J. M., Favus, M., Lombardi, A., et al. Comparative study of alendronate versus etidronate for the treatment of Paget's disease of bone. J Clin Endocrinol Metab 81:961–967; 1996.

119. Siris, E. Goals of treatment fo Paget's disease of bone. J Bone Miner Res 14(Suppl. 2):49–52; 1999.

120. Siris, E. S., Canfield, R. E., Jacobs, T. P., Stoddart, K. E., and Spector, P. J. Clinical and biochemical effects of EHDP in Paget's disease of bone: Patterns of response to initial treatment and to long-term therapy. Metab Bone Dis Rel Res 3:301–308; 1981.

121. Siris, E. S., Chines, A. A., Altman, R. D., Brown, J. P., Johnston, C. C. Jr., Lang, R., et al. Risedronate in the treatment of Paget's disease of bone: An open label, multicenter study. J Bone Miner Res 13:1032–1038; 1998.

122. Sochart, D. H. and Porter, M. L. Charnley low-friction arthroplasty for Paget's disease of the hip. J Arthroplasty 15:210–219; 2000.

123. Solomon, L. R., Evanson, J. M., Canty, D. P., and Gill, N. W. Effect of calcitonin treatment on deafness due to Paget's disease of bone. Br Med J 2:485–487; 1977.

124. Steinbach, H. L. Some roentgen features of Paget's disease. Am J Radiol 86:950–964; 1961.

125. Stone, M. D., Hawthorne, A. B., Kerr, D., Webster, G., and Hosking, D. J. Treatment of Paget's disease with intermittent low-dose infusions of disodium pamidronate (APD). J Bone Miner Res 5:1231–1235; 1990.

126. Thiebaud, D., Jaeger, P., and Burckhardt, P. Paget's disease of bone treated in five days with AHPrBP (APD) per os. J Bone Miner Res 2:45–52; 1987.

127. Thiebaud, D., Jaeger, P., Gobelet, C., Jacquet, A. F., and Burckhardt, P. A single infusion of the bisphosphonate AHPrBP (APD) as treatment of Paget's disease of bone. Am J Med 85:207–212; 1988.

128. U.S. Department of Health and Human Services. Agency for Health Care Policy and Research. Acute pain management: Operative or medical procedures and trauma. Clinical Practice Guideline No. 1. AHCPR Publication #92-0023; 1993; 107.

129. Vega, E., Gonzalez, D., Ghiringhelli, G., and Mautalen, C. Intravenous aminopropylidene bisphosphonate (APD) in the treatment of Paget's bone disease. J Bone Miner Res 2:267–271; 1987.

130. Vega, E., Mautalen, C., Roldan, E. J., and Perez-Lloret, A. Preliminary study of multiple increasing oral doses of dimethyl-APD on bone metabolism dynamics and safety profile. Drugs Exp Clin Res 20:103–108; 1994.

131. Vellenga, C. J., Pauwels, E. K., Bijvoet, O. L., Harinck, H. I., and Frijlink, W. B. Quantitative bone scintigraphy in Paget's disease treated with APD. Br J Radiol 58:1165–1172; 1985.

132. Wallace, E., Wong, J., and Reid, I. R. Pamidronate treatment of the neurologic sequelae of pagetic spinal stenosis. Arch Intern Med 155:1813–1815; 1995.

133. Watts, R. A., Skingle, S. J., Bhambhani, M. M., Pountain, G., and Crisp, A. J. Treatment of Paget's disease of bone with single dose intravenous pamidronate. Ann Rheum Dis 52:616–618; 1993.

134. Weisz, G. M. Lumbar spinal canal stenosis in Paget's disease. Spine 8:192–198; 1983.

135. Wellman, H. N., Schauwecker, D., Robb, J. A., Khairi, M. R., and Johnston, C. C. Skeletal scintimaging and radiography in the diagnosis and management of Paget's disease. Clin Orthop Rel Res 127:55–62; 1977.

136. Wilner, D. and Sherman, R. S. Roentgen diagnosis of Paget's disease (osteitis deformans). Med Radiogr Photogr 42:35–78; 1966.

137. Wimalawansa, S. J. Long- and short-term side effects and safety of calcitonin in man: A prospective study. Calcif Tissue Int 52:90–93; 1993.

138. Yap, A. S., Mortimer, R. H., Jacobi, J. M., Galligan, J. P., Perry-Keene, D. A., and Khafagi, F. A. Single-dose intravenous pamidronate is effective alternative therapy for Paget's disease refractory to calcitonin. Horm Res 36:70–74; 1991.

139. Yates, A. J., Percival, R. C., Gray, R. E., Atkins, R. M., Urwin, G. H., Hamdy, N. A., et al. Intravenous clodronate in the treatment and retreatment of Paget's disease of bone. Lancet i:1474–1477; 1985.

Paget Disease: A Review of Current Knowledge[1]

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An overview of the current knowledge on Paget disease is presented. Recent advances include a better understanding of its etiology, pathogenesis, and effects on bone structure and function. The clinical and radiologic features are discussed, as are some new ideas on treatment protocol. The therapeutic agents considered include calcitonin, disodium etidronate, and mithramycin.

PAGET disease is a condition commonly detected among the elderly. Its presentation varies from a painful or deforming skeletal affliction to an asymptomatic disorder diagnosed on routine clinical, biochemical, or radiologic assessment. Recent advances have improved our understanding of this disease—its etiology, pathogenesis, and effects on bone structure and function. At the same time, new therapeutic agents offer both symptomatic relief and some control of the basic disease process. A conference devoted to the discussion of current concepts and new knowledge in Paget disease was held in March 1980, sponsored by the Kroc Foundation (1).

PATHOPHYSIOLOGY

Considerable interest has centered on the origin of the bone cells involved in the disordered bone remodeling in Paget disease. The osteoclast which plays an important role in the initial phase of accelerated bone resorption appears to be derived from a hemopoietic stem cell and probably shares a common ancestry with the macrophage and monocyte. The osteoblast, which generates structurally abnormal new bone, arises from a non-migratory connective tissue stromal cell.

Evidence from at least two laboratories suggests a viral etiology for Paget disease. The evidence is based on immunohistology and the finding, on electron microscopy, of intranuclear viral inclusions within pagetic osteoclasts. In one laboratory, immunohistologic studies suggest a role for measles virus, while in the other, tagged immunofluorescent antibodies implicate respiratory syncytial virus (RSV). Viral cultures, however, have so far yielded negative results. In time, Paget disease may be classified among the slow virus infections. It is postulated that the long-standing presence of virus in bone cells leads to the fusion of infected with non-infected cells, resulting in the large, multinucleated osteoclasts characteristic of the initial changes in Paget disease. Experience in the management of Paget disease suggests that all the areas of ultimate

bony involvement are probably already affected at the time of the initial radiologic diagnosis. While serial pathologic and radiologic changes do occur in a single involved bone over a period of time, the appearance of new areas of involvement in additional bones is unusual. This observation is compatible with the suggestion that an infective agent such as a slow virus has invaded different bones at approximately the same time.

The altered bone remodeling is mediated by osteoclasts and osteoblasts seemingly coupled to act as basic "bone remodeling units" (BRU's). In Paget disease, there is a marked acceleration in the production rate of these basic BRU's, leading to an increase in the number and activity of these bone cells. Paget disease is initiated by an intense wave of osteoclastic activity, with resorption of normal bone by giant, multinucleated cells. Normal lamellar bone is resorbed in a haphazard fashion, resulting in bizarre and irregularly-shaped resorption cavities. After a variable period of time, osteoblastic resorption is replaced by a vigorous osteoclastic response which causes exuberant formation of primitive or woven bone in association with increased vascularity and a pronounced connective tissue reaction. Some of the woven bone may eventually be replaced by normal-appearing lamellar bone. The localized areas of disorganized resorption cavities with intense new bone formation lead to a distorted bony trabecular pattern, the so-called "mosaic pattern". This is due to the occurrence of irregular cement lines resulting from uncontrolled bone resorption. Subperiosteal new bone may form and increase the diameter of the skeleton in the involved area.

Bone biopsy studies reveal that well-defined histologic signs of the disease can sometimes be identified in bones which appear normal both clinically and radiologically. Moreover, bone histomorphic evidence points to an increase in trabecular resorption surfaces and osteocyte lacunar size in otherwise uninvolved bones, which suggests an increased parathyroid hormone effect. It is proposed that increased calcium demands in the enlarging

pagetic bone lead to secondary hyperparathyroidism and increased bone remodeling in normal bones. Secondary hyperparathyroidism in patients with Paget disease is further suggested by the finding of depressed serum calcium levels in association with increased serum immunoreactive parathyroid hormone levels (iPTH) and increased urinary excretion of nephrogenous c'AMP in some patients. Nephrogenous c'AMP reflects renal tubular secretion of c'AMP and is an indirect measurement of biologically active PTH.

The increase in bone remodeling in Paget disease allows the utilization of bone biochemical markers, such as calcium, and products of collagen metabolism in blood and urine as parameters of disease activity. Calcium turnover is markedly increased. Ions moving in and out of the skeleton at rates often over 20-fold normal; yet this is associated with only minimal or no change in total calcium balance and serum concentrations of mineral ions. Bone mineral that is released by osteoclastic action is promptly redeposited in areas of osteoblastic new bone formation. Occasionally, as a result of fracture and immobilization, more calcium may be released from the skeleton than is reutilized. Under these circumstances, urinary calcium and, more rarely, serum calcium, may increase (so-called immobilization hypercalcemia).

Methods for studying collagen metabolism have improved, so that chemical markers of both collagen synthesis and degradation can now be measured. Type I collagen molecules released by the action of osteoclasts are denatured and then cleaved by proteases produced by the osteoclasts. The dialyzable peptide fragments that result from this process contain amino acid markers such as 4-hydroxyproline, hydroxylysine, and hydroxylysine glycosides. These can be measured in the urine and, in general, reflect the degree of collagen breakdown. In contrast, peptides detected in the urine that are non-dialyzable are better correlated with collagen synthesis. Serum isoenzymes for alkaline phosphatase of skeletal origin also reflect the extent of new bone formation. Occasionally, the serum alkaline phosphatase level is normal, as, for example, in patients with monostotic involvement, or late in the disease when the pathologic process may appear to be quiescent. The serum acid phosphatase level may also be increased on occasion and probably reflects altered osteoclastic activity. Recently, a non-collagenous bone-protein containing gamma carboxy-glutamic acid was found in increased levels in the serum of patients with Paget disease. Paget disease represents a human laboratory for the continuing study of chemical substances involved in bone resorption and formation.

CLINICAL FEATURES

The majority (>90%) of patients with Paget disease are asymptomatic. Pain of a deep-seated nature can occur and may be associated with bony deformity and pathologic fracture. Pain may result from stretching of the periosteum and from hyperemia. Several varieties of headache due to involvement of the skull have been described. The extent of the pain is not necessarily related to the degree of radiologic involvement and its cause is frequently difficult to differentiate from that of the pain in degenerative arthritis which often occurs in joints contiguous to involved bones. The skin overlying the bone may be erythematous and warm. Any bone in the skeleton may be involved, but the most common sites are the skull, spine, pelvis, femur, and tibia. An enlarged skull, dorsal kyphosis, and bowing of the legs are the classic signs of advanced disease.

New bone may impinge upon nerves, causing cranial nerve palsies. This may lead to optic atrophy and diminished auditory acuity as well as other compressive effects. Involvement of the base of the skull may cause brain stem symptomatology or may produce basilar invagination great enough to interfere with flow of cerebrospinal fluid, resulting in "normal-pressure" hydrocephalus. Paget disease affecting the vertebral bodies and spinal foramina may cause narrowing of the spinal canal with pressure on the spinal cord, cauda equina, and nerve roots, and result in marked neurologic symptoms.

Occasionally, increasing local pain and rising levels of serum alkaline phosphatase signal the complication of osteogenic sarcoma, which develops in about 0.15% of patients. Such lesions may arise simultaneously in multiple sites in involved bones. The lesions are most common in the humerus and skull, less common in the pelvis and femur, and they rarely occur in the lumbar spine. When extensive disease is present, high-output cardiac failure may occur; however, this is uncommon in the absence of some other primary cardiac disease.

Pathologic fractures, especially of the femur and tibia, are a frequent complication. In the majority of cases, fractures heal promptly, but nonunion occurs in up to 10% of cases.

RADIOLOGIC FEATURES

Radiologic changes in the skeleton are usually distinctive in Paget disease, but confusion in diagnosis can occur. The initial lytic phase may be seen as (a) a discrete radiolucent area (osteoporosis circumscripta in the skull), (b) a typical V-shaped resorption front, or (c) a smaller, flame-shaped lytic lesion in the tibia or other long bone. In most instances, mixed lytic and sclerotic phases are observed in the same general area of osseous involvement. The bony trabecular bundles and cortex may become thickened and irregular. Subperiosteal new bone may form and increase the external diameter of the involved bone. Single or multiple fissure-fractures may occur on the convex surface of an involved femur or tibia and may superficially resemble Looser zones, like those observed in osteomalacia.

Isotope bone scanning is more sensitive than skeletal radiography in detecting the early stages of the disease. It should be utilized for screening purposes when Paget disease is suspected, as, for example, when an elderly patient has an unexplained elevated level of alkaline phosphatase detected by automated chemical screening.

Technetium diphosphonate radionuclide scanning provides information on both extent and current activity of the disease. Early studies with gallium-67 suggest this may be a more sensitive bone scanning agent in Paget disease, but data on its use are only preliminary, and consideration of increased cost and patient inconvenience make routine use as yet impractical.

DIAGNOSIS

The differential diagnosis of Paget disease is not usually difficult. A patient may present with an unexpected elevation of alkaline phosphatase detected on multichannel chemical screening of the serum. Isoenzyme fractionation may then reveal the skeletal fraction to be increased. If "hot spots" appear on a technetium bone scan, they may correspond to areas of radiologically identifiable Paget disease. On the other hand, the radiologic appearance may suggest osteoblastic metastatic bone disease; however, increased external dimensions of the bone will favor the diagnosis of Paget disease. Osteitis fibrosa, due to hyperparathyroidism, may simulate the radiologic appearance of the skull in Paget disease, but differentiation between these two conditions will usually be possible from the biochemical findings. There is evidence of an increased prevalence of primary hyperparathyroidism among patients with Paget disease.

MEDICAL TREATMENT

New and effective therapeutic agents have been recently introduced. Following administration, skeletal pain decreases and the serum alkaline phosphatase and urinary hydroxyproline levels, which represent measures of active bone remodeling, are lowered. Since most patients are asymptomatic, it is often difficult to decide whether pain is due to Paget disease or associated degenerative arthritis which frequently occurs in contiguous joints, particularly those of the spine, hips, and knees. On occasion, both conditions contribute to the patient's discomfort. It is probable, in fact, that the arthritis results not only from altered bone mechanics and gravitational forces, but also from a disturbance of enchondral bone formation; this disturbance may be related to increased vascularity of subchondral bone which is associated with the Paget disease itself.

Calcitonin was the first of the newer medications found to be effective in treating Paget disease. Following its use, pain is reduced as are serum alkaline phosphatase and urinary hydroxyproline excretion. Antibodies to the more commonly used salmon calcitonin may occur but may not necessarily interfere with its therapeutic effect. Synthetic human calcitonin, which is not normally associated with antibody formation, is available in some centers. Following administration, osteoclastic activity decreases. Both histologic and radiologic evidence suggest that bone which appears normal can form after the administration of calcitonin. Moreover, the temperature of the overlying skin may decrease, and high-output cardiac failure may im-

prove. Other indications for the use of calcitonin include immobilization hypercalcemia, repeated fractures or nonunion, and neurological lesions. Administration of calcitonin is also indicated prior to elective orthopedic surgery on bones involved by Paget disease. Relief of pain generally occurs in a matter of weeks, but the beneficial effects of treatment may not be fully evident until six months after commencement of therapy. Following therapy, neurologic compressive features involving the cranial nerves, spinal cord, and nerve roots may regress. Synthetic salmon calcitonin is usually given as 50–100 MRC (Medical Research Council) units subcutaneously three times a week. For historical reasons, salmon calcitonin dosage is expressed in terms of units of biological activity, 100 MRC units being equivalent to 1 mg of the newer human calcitonin preparations. The main disadvantage is that calcitonin has to be given by injection. Thirty per cent of patients report transient feelings of flushing, warmth, or nausea immediately following treatment. Nevertheless, there do not appear to be any serious local or systemic side effects.

Another agent recently introduced for the treatment of Paget disease is disodium etidronate, a diphosphonate. Diphosphonates cover bone surfaces and inhibit bone resorption and formation. They are not subject to enzymatic degradation from pyrophosphatases. Too large a dose will interfere with mineralization and may result in osteomalacia and an increase in the frequency of pathologic fractures. Disodium etidronate (EHDP, Didronel), the diphosphonate most commonly used, results in a decrease in skeletal pain as well as reductions in alkaline phosphatase and urinary hydroxyproline levels in some 60% of patients. The recommended dose is 5 mg/kg body weight per day, given orally for periods of six months. No long-term deleterious effects have been reported with administration of the recommended dose. Recently, favorable experience has been reported with the use of other diphosphonates such as dichloromethylene diphosphonate (Cl_2 MDP), which displays a greater inhibitory effect on bone resorption and only minimal inhibition of bone mineralization.

Mithramycin, an anti-tumor agent produced by *Streptomyces* and which is used in the treatment of embryonal tumors of the testis and in resistant hypercalcemia, has been shown to decrease osteoclastic bone resorption. When given intravenously in short courses of 25 mcg/kg per day, bone pain is decreased and both serum alkaline phosphatase and urinary hydroxyproline excretion fall. Following administration there have been reports of transient elevations of hepatic enzymes in the serum, reduction in the peripheral platelet and leucocyte counts, and renal impairment. Because of its potential toxicity, mithramycin should perhaps be reserved for resistant cases or for special indications.

Administration of mithramycin may be indicated in the young patient with deforming Paget disease in whom an aggressive attempt at long-term remission is sought. Another special indication may be in a case where confusion exists as to whether pain is arising from bone involved with

Paget disease or from an adjacent arthritis. The former case may require repeated therapeutic courses of several days' duration. However, in the latter case, because of the rapidity with which mithramycin appears to control the pain of Paget disease, a single one-day course may suffice as both a diagnostic and a therapeutic maneuver by relieving pain as well as improving the biochemical derangements.

If a case proves resistant to therapy with either calcitonin or a diphosphonate, it is reasonable to consider simultaneous administation of these agents. In those cases which have proved resistant to treatment with calcitonin and diphosphonate given separately and in combination, favorable results and a few long-term remissions have been achieved with courses of mithramcyin alone.

The response to therapy should be monitored by assessing improvements in the symptomatology, the biochemical parameters (especially alkaline phosphatase) and, in the case of extensive or severe disease, the bone scan obtained after approximately six months of therapy.

Paget disease should be treated when it causes skeletal pain and tenderness or when there are neurologic symptoms; compressive fractures, marked deformities, or other complications. In patients with extensive radiologic involvement but no symptoms, the role of medications in preventing future fractures and complications is unclear.

The judicious introduction of therapy would appear reasonable, but data regarding the efficacy of such an approach are not yet available. When evidence of severe involvement is present early in life, the chances of marked deformities and complications developing are high, and such sequelae might well be prevented by early, and perhaps intermittent, therapy.

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REFERENCES

1. Altman RD, Singer F (eds). Proceedings of the Kroc Foundation Conference on Paget's Disease of Bone. Arthritis Rheum 1980; 23:No. 10.
2. Broadus AE, Mahaffey JE, Bartter FC, Neer RM. Nephrogenous cyclic adenosine monophosphate as a parathyroid function test. J Clin Invest 1977; 60:771–783.
3. Goldman AB, Bullough P, Kammerman S, Ambos M. Osteitis deformans of the hip joint. AJR 1977; 128:601–606.
4. Rao DS, Feigelman T, Marel G, et al. Evidence for secondary hyperparathyroidism in Paget's disease of bone. (in press)
5. Singer FR. Paget's disease of bone. New York: Plenum Medical, 1977.
6. Waxman AD, McKee D, Siemsen JK, Singer FR. Gallium scanning in Paget's disease of bone. Effect of calcitonin. AJR 1980; 134: 303–306.

Review Article

Paget Disease of Bone: Current Status and a Look Back to 1943 and Earlier

Donald Resnick[1]

Paget disease of bone (osteitis deformans) is a condition of unknown cause that affects approximately 3–4% of the population over the age of 40 years [1, 2]. Its name is a tribute to Sir James Paget, an astute clinician whose original description of the disease in 1877 [3] serves as a model to those of us who strive to write with clarity and precision. In that description, Paget presented a detailed report of one patient whom he had observed for approximately 20 years, as well as a brief summary of several other individuals with the same disorder. Although other reports preceded his, Paget's account is recognized as a lesson in accurate and lucid writing, and the disorder is now called Paget disease.

During the 110 years that have elapsed since Paget's landmark article, the disease has been the subject of intense interest, speculation, and investigation. It now is known that Paget disease shows certain geographic and racial characteristics: It is especially common among inhabitants of Great Britain, Australia, and certain areas of continental Europe; it is relatively frequent in the United States but rare in Japan and China. Paget disease predominates in middle-aged and elderly individuals, although reports document its occasional occurrence in young adults [4]. It affects men more often than it affects women, and the age of onset may be slightly lower in men than in women [5]. Familial history of the disease has been identified in some instances, with reports indicating that several members of a family in various generations and identical twins may be affected [6]. Although variable in its clinical

manifestations, Paget disease is asymptomatic in most instances. The disorder is characterized by excessive and abnormal remodeling of bone. It passes through active and quiescent phases leading to a combination of osseous resorption and apposition that produce a distinctive pathologic and radiographic appearance in which irregular bone fragments have a thickened and disorganized trabecular (mosaic) pattern (Fig. 1).

A reprint of the report by Groh [7], which appeared in the AJR 45 years ago, precedes this paper and underscores the interest that radiologists have had in Paget disease. In Groh's report, he describes nine patients, seven men and two women between the ages of 35 and 63 years, with "localized" involvement of the spine (six patients), innominate bone (two patients), or tibia (one patient). He further comments on the causes and natural history of the disease and the value of the radiographic and laboratory examination in studying its course; in the form of an addendum, Groh notes three other cases in which monostotic involvement was observed. The article is interesting reading and shows the modern radiologist what was known or believed about Paget disease more than 4 decades ago. The information in this paper provides an appraisal of Groh's report and a summary of current concepts of the disease. These are supplemented with quotes derived from the original descriptions of Paget, many of them more than 100 years old, which will emphasize the fact that accurate scientific writing will long be valued.

It is often said or implied that, in our profession, a man cannot be both practical and scientific; science and practice seem to some people to be incompatible. Each man, they say, must devote himself to the one or the other. The like of this has long been said, and it is sheer nonsense.

Sir James Paget (1894) [8]

Etiology

"With regard to the nature of the process . . . only three things could produce so great an increase in the size of a bone, namely, new growth (tumor), hypertrophy, and chronic inflammation. The first of these may be at once set aside as out of the question, nor is the second much more probable than the first. . . . of the three causes chronic inflammation alone remains."

Sir James Paget (1877), quoting Mr. Butlin [8]

Despite the scrutiny to which Paget disease has been subjected, there still is no consensus about its cause. It has been suggested that Paget disease is an inflammatory, hereditary, neoplastic, vascular, traumatic, endocrinologic, or immunologic disorder [2]. Until recently, the evidence supporting any specific cause has been either nonexistent or inconstant and easily refuted [9]. For example, reports of familial aggregation of cases and of increased prevalence among certain ethnic groups have lead some investigators to argue that an autosomal dominant pattern of inheritance is present, whereas other investigators have emphasized the fundamental role that parathyroid hormone plays in the development of Paget disease. An inflammatory cause has been championed by numerous investigators, including Paget. Indeed, Groh [7], at the outset of his discussion, states in no

uncertain terms that the pagetic lesion must be infectious in nature, citing clinical manifestations consistent with infection (such as redness, swelling, and a localized increase in heat) that repeatedly have been described.

In agreement with Paget's original hypothesis and with Groh's strong belief, the theory that the cause is infectious, specifically a viral illness, has gained support by observations in a number of recently published articles [10]. Active pagetic bone is characterized by the presence of giant osteoclasts containing large numbers of nuclei. In 1974, Rebel et al. [11] identified, by ultrastructural examination, organized groups of microcylinders within the nuclei and the cytoplasm of the osteoclasts in areas of bone affected with Paget disease. These intranuclear inclusion bodies, the presence of which have been corroborated in subsequent investigations [12–14], are not observed in osteoblasts or osteocytes in pagetic bone or in osseous tissue derived from patients with a variety of other skeletal disorders, even those characterized by osteoclastic proliferation [2]. The inclusions, however, are not entirely specific for pagetic bone, having been identified within giant cell tumors in patients with [15, 16] or without [17, 18] Paget disease. Ultrastructural characteristics of the pagetic osteoclasts led to the hypothesis that the inclusions have a viral nature. Similar cellular characteristics are observed in disorders produced by certain viruses, specifically subacute sclerosing panencephalitis related to a *Paramyxovirus* of the measles group [19]. Additional morphologic evidence for a viral cause of the intranuclear inclusions has included (1) the dense fibrillar material associated with some of the inclusions is similar to that found in the nuclei of virus-infected cells; (2) filament bundles and spindle-shaped structures enclosed in double membranes observed in the cytoplasm of some osteoclasts are considered an indirect cellular response to viral attacks; and (3) the presence of enormous osteoclasts in pagetic bone is compatible with abnormalities of in vitro measles virus infection [20]. Certain immunologic data have reinforced this viral hypothesis [13, 21], and significant and sustained viral antibody titers against the measles virus have been detected in a few patients with Paget disease. Although the cause of the disease still is not entirely clear, all of the experimental evidence cited above would appear to support the concept that Paget disease is a slowly developing viral infection of osteoclasts.

Skeletal Distribution and Extent

The most frequent seats of the osteitis have been . . . the tibiae, femora, clavicles, spine and vault of the skull . . . The skull became gradually larger, so that nearly every year, for many years, his hat and the helmet that he wore as a member of a Yeomanry Corps needed to be enlarged . . . The length of the spine thus seemed lessened, and from a height of six feet one inch, he sank to about five feet nine inches. . . .

Sir James Paget (1877, 1889) [8]

Fig. 1.—Histologic abnormalities of Paget disease: mosaic pattern. Note presence of cement lines (arrow) joining areas of lamellar bone.

The predilection of Paget disease to affect the axial skeleton is well documented. Particularly characteristic is the involvement of the innominate bone (20-75% of cases), sacrum (30-60%), spine (30-75%), and skull (25-65%). Additionally, the proximal long bones, particularly the femur (25-35% of cases), are commonly affected. A preference for the lower extremities and a tendency for right-sided alterations are other emphasized features of the distribution of Paget disease. The central distribution corresponds to that of hematopoietic red marrow, so that the locations of Paget disease are similar to those of skeletal metastases; however, the relative frequency of tibial involvement and infrequency of rib involvement are features of Paget disease not shared by osseous metastases [2].

Although these general characteristics of Paget disease are well accepted, accurate assessment of the precise distribution of the disease is difficult owing to the facts that osseous lesions are commonly asymptomatic and that a true delineation of the extent and sites of involvement requires an imaging survey of the entire skeleton. Although Paget disease is polyostotic in most cases, it may be initially or totally monostotic in 10-35% of cases. In Groh's description of nine patients with monostotic abnormalities [7], radiographic surveys of classic target areas of the disease were used to verify the absence of involvement at other skeletal sites. At present, the assessment of the extent of skeletal disease might be accomplished better with bone scintigraphy (Fig. 2). As would be expected, areas of osseous disease are depicted as sites either of increased uptake of bone-seeking radiopharmaceutical agents or, in appropriate locations, of marrow replacement [22-25]. The immediate accumulation of radionuclide reflects an increased blood flow to bone, which may explain false-positive brain scintiscans in patients with calvarial Paget disease [26]. Scintigraphic abnormalities may precede radiographic changes, underscoring the greater sensitivity of radionuclide vs radiologic examinations. Furthermore, scintigraphy
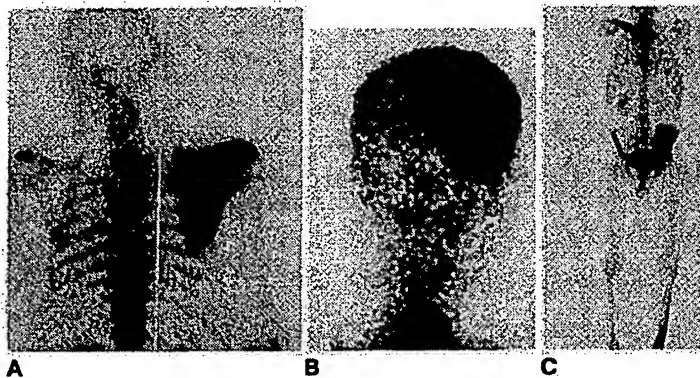
can document the extent of the initial, osteolytic phase of Paget disease [27], during which increased radionuclide activity may be particularly prominent at the advancing edge of bone lysis, corresponding to sites of intense vascularity, osteoclastosis, and new bone formation [28]. Serial bone scintigraphs may provide objective evidence of the effects of various therapeutic agents (see later discussion).

As noted by Groh [7], monostotic Paget disease appears to predominate in the axial skeleton, although any portion of the skeleton may represent the sole site of involvement. Although Paget disease confined to one bone or a portion of one bone may be more difficult to diagnose, characteristic radiographic findings generally are observed, allowing accurate differentiation from other conditions. In the tubular bones, a predilection for the epiphyseal area, an advancing wedge of radiolucency (Fig. 3), periosteal apposition of new bone leading to widening of the osseous surface, and deformity and fracture are characteristic, although not invariable, features of Paget disease. Rarely, narrowing or contraction of the involved bone is seen [29]. Diaphyseal changes without epiphyseal abnormality (Fig. 4) are extremely unusual but may be observed, particularly in the tibia [30, 31]. Furthermore, in the tibia, the proximal extent of disease may be located not in the epiphysis but in the anterior tibial tubercle (Fig. 5) [32].

Monostotic involvement of the spine, as was evident in six of the nine patients reported by Groh [7], is not difficult to diagnose when typical radiographic features, including the picture-frame vertebral body, are observed (Fig. 6). Groh's description of a second pattern of vertebral involvement, in which more generalized abnormality leads to an ivory vertebral body, is an important observation that may alert the modern radiologist that Paget disease of the spine can simulate lymphoma or skeletal metastases. Further, as illustrated in Groh's article, collapse of an affected vertebral body may complicate accurate diagnosis unless pagetic changes in the remaining portions of the vertebra are identified.

Fig. 2.—Scintigraphy shows extent of skeletal involvement. Intense uptake of bone-seeking radiopharmaceutical agent occurs in each case.
A, Scapula.
B, Cranial vault.
C, Clavicle, innominate bone, and tibia.
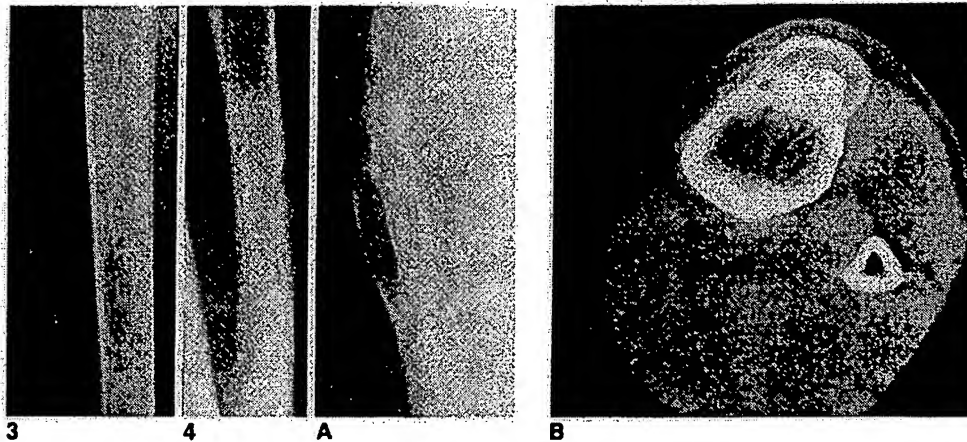
A   B   C

Fig. 3.—Osteolytic phase: tubular bone. Radiolucent wedge in diaphysis is well-defined.

Fig. 4.—Diaphyseal involvement of radius. Involvement of this segment of bone without epiphyseal involvement is distinctly unusual.

Fig. 5.—Involvement of the anterior tibial tubercle.
A, Lateral radiograph reveals osteolytic focus of Paget disease extending shaftward from region of tibial tuberosity.
B, Transaxial CT confirms the intracortical location of process.
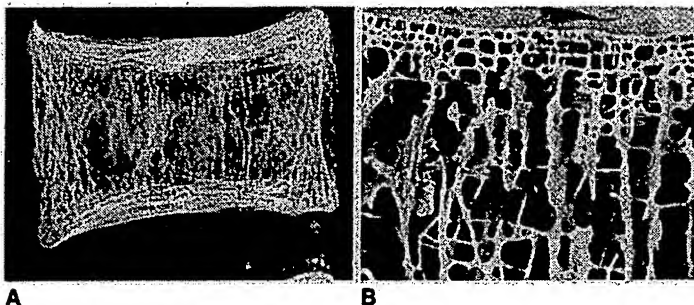(Courtesy of H. Rodriguez, Santa Barbara, CA.)

Fig. 6.—Picture-frame vertebral body in Paget disease.
A and B, Radiograph (A) and photograph (B) of a macerated vertebral body show condensation of trabeculae at periphery of vertebral body that is characteristic of disease process.

Natural History and Effect of Therapy

The primary event in Paget disease is intense focal bone resorption that is followed by disorderly bone formation, although the coupling of bone resorption and formation in this disease shows considerable variation from one patient to another and, indeed, from one involved skeletal region to another or from one period of time to another in a single individual [33]. The resulting aberrations in normal bone remodeling may lead to variable radiographic appearances,

depending on the relative contributions of osseous resorption and apposition within the periosteal and endosteal cortical envelopes [34]. An increased or decreased external bone contour and a narrowed or enlarged marrow cavity may be observed, in sharp contrast to the bone expansion that was long regarded as a universal feature of the disease.

Paget disease generally is considered a relentlessly progressive disorder resulting in the conversion of normal uninvolved bone to abnormal bone, and, ultimately, in involvement of the entire bone. This concept was supported by Groh [7], who indicated the variability in the rate of disease progression.

Numerous investigations have confirmed that variation exists in the rate of conversion of normal to pagetic bone, although, in opposition to Groh's belief that rapid progression might be encountered, most studies have documented a slow, gradual extension of disease. The rate of progression in Paget disease generally has been estimated to be between 7 and 16 mm per year [10, 33, 35, 36]. This relatively slow rate of conversion of normal to diseased bone, combined with the three-dimensional nature of the process, accounts for the common reporting of "unchanged" disease extent on serial radiographs, and also explains, in part, the popularity of scintigraphy as an effective method of monitoring the extent (and activity) of the disease.

The absence of any reference to the treatment of Paget disease in Groh's report is understandable, because effective therapeutic agents in this disease have been available only since the 1950s. One such agent is calcitonin, a potent inhibitor of bone resorption, which can lead to relief of pain for patients with Paget disease within weeks of its administration and to a reduction in serum levels of alkaline phosphatase and urinary levels of hydroxyproline. Although the rate of extension of the disease in pagetic patients being treated with calcitonin has been reported to be unchanged when compared with that rate in untreated patients [33], radiographic improvement in the appearance of the pagetic bone during such treatment has been recognized by numerous investigators; such improvement is evident in both cortical and trabecular bone, especially during the osteolytic phases of the disease [37–40]. After the therapy is stopped, a period of rapid bone resorption, even greater than that typical of untreated Paget disease, has been observed [37].

A second agent used in the therapy of Paget disease is disodium etidronate (EHDP), a diphosphonate. Diphosphonates inhibit bone resorption and mineralization by binding to the hydroxyapatite crystals and inhibiting their growth and dissolution [2]. Although, again, an unchanged rate of pro-

Fig. 7.—Scintigraphic assessment of osseous response to treatment.
A–C, Bone scans obtained 1 year apart show (A) initial intense accumulation of the radionuclide in tibia with partial resolution (B and C) over 2-year period during treatment with thyrocalcitonin. Residual activity in C corresponded in position to sites of insufficiency fractures.
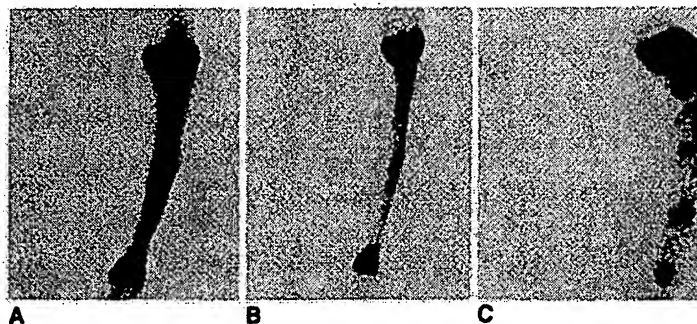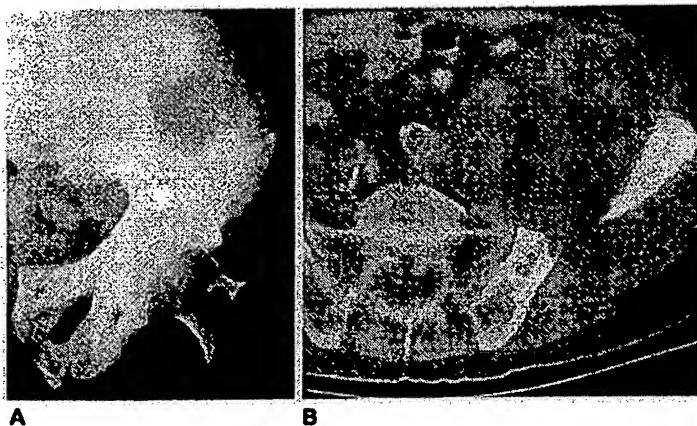

Fig. 8.—Sarcomatous transformation of Paget disease in innominate bone.
A and B, Radiograph (A) and CT scan (B) show osteolytic lesion in pagetic ilium with large soft-tissue mass. (Courtesy of P. Kaplan, Omaha, NE.)
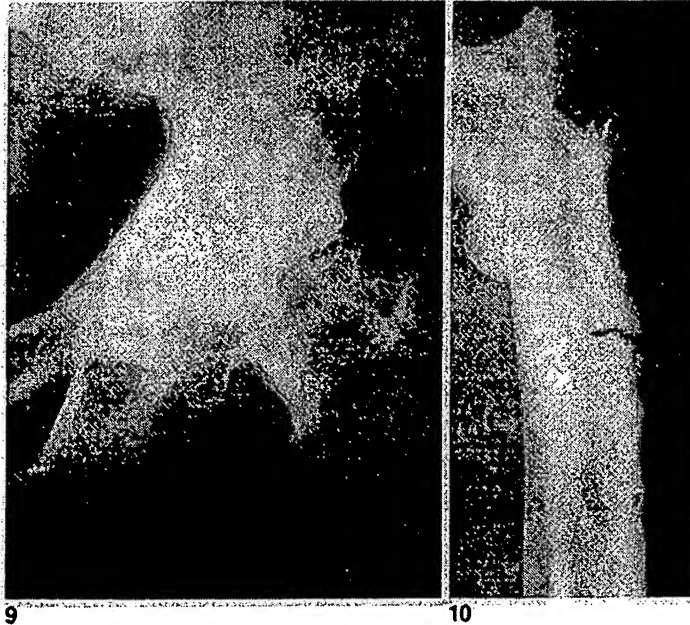
Fig. 9.—Osteoarthritis of hip in Paget disease. Joint space narrowing involving superior and axial portions of hip is evident. Pagetic changes are observed in ilium.

Fig. 10.—Fractures in Paget disease. Numerous insufficiency fractures are evident in lateral cortex of femur, and one extends medially, partially across bone. (Courtesy of G. Greenway, Dallas, TX.)

gression of Paget disease during EHDP therapy has been observed in some patients [33]. In most patients with the disease, such therapy results in a decrease in skeletal pain, a reduction in serum alkaline phosphatase and urinary hydroxyproline levels, and (based on evaluation of material derived from bone biopsies) a conversion of pagetic bone to a more normal tissue [41–45]. However, a number of reports have indicated an increased frequency of fractures in patients with Paget disease who have been treated with EHDP [46, 47], a complication that may relate to drug dosage and to the occurrence of osteomalacia [47, 48]. Other diphosphonates have been suggested as therapeutic alternatives to EHDP [49] and one, 3-amino-1-hydroxypropilidene-1, 1-diphosphonate (ADP), has been reported to significantly decrease the rate of disease progression [33].

Mithramycin is an antibiotic with cytotoxic activity that has been used successfully in the treatment of Paget disease, leading to a decrease in bone pain and an improvement in laboratory parameters of disease activity [2]. Assessment of the skeletal disease by radiographic and scintigraphic techniques during therapy also has indicated a return to a more normal situation [50], although radiologic change is less frequent and dramatic after the use of mithramycin than after calcitonin treatment. Owing to its significant toxicity, mithramycin administration is best reserved for cases of Paget disease that are resistant to other forms of treatment [51].

Although radiographic improvement may be observed in the appearance of skeletal involvement in Paget disease during treatment with any of the aforementioned therapeutic agents, the changes usually are subtle and dependent on the stage of the disease at the time of the treatment; also, these changes include a conversion of osteolytic lesions to osteosclerotic ones, consolidation of the layered new bone, reappearance of a cortex of uniform density, restoration of corticomedullary differentiation, widening of the medullary canal, thinning of the cortex, a reduction in the external volume of the bone, and a return to normal osseous shape [2]. In monitoring the response of pagetic bone to any of these therapeutic agents, scintigraphy can show a distinct decrease in radionuclide accumulation in diseased areas as a marker of a beneficial therapeutic response (Fig. 7) [52, 53]. On scintigraphy, recurrence of the disease is typically accompanied by a rise in activity in one or more bones or by spread of disease into an adjacent normal bone. When these changes are present, they precede biochemical indicators of recurrent disease.

Complications

... As he was riding and suddenly raised his arm the bone broke near the shoulder ... and I amputated the arm at the shoulder joint.

In three out of the five well-marked cases that
I have seen or read-of cancer appeared late in
life ... suggesting careful inquiry.

Sir James Paget (1877) [8]

The musculoskeletal complications of Paget disease in-
clude osseous deformities, fractures, neoplasms, soft-tissue
masses, osteomyelitis, extramedullary hematopoiesis, crystal
deposition (monosodium urate, hydroxyapatite and, possibly,
calcium pyrophosphate dihydrate), neurologic abnormalities,
and degenerative joint disease. Of these, Groh [7] provided
examples of deformity of the tibia (leading to anterior bowing
of the bone) and innominate bone (producing acetabular
protrusion and joint-space diminution in the hip) and com-
mented on nerve-root compression related to vertebral in-
volvement. Spinal stenosis as a complication of Paget disease
of the vertebral column has been confirmed subsequently in
a number of investigations, especially those in which CT has
been used to evaluate the extent of bone involvement [54–
57]. Compression of the spinal cord can be due to expansion
of bone from the pagetic process, collapse with hemorrhage,
deformity, vascular compromise, or neoplastic degeneration;
additional neurologic dysfunction in Paget disease may be
related to changes in the base of the skull. Cauda equina
compression in this disease has also been attributed to ossi-
fication of extradural fat [58], and the sciatic nerve may be
compressed either in external rotation between an enlarged
ischium and lesser trochanter of the femur or in internal
rotation between the ilium and piriformis [59].

Neoplastic involvement in Paget disease includes sarcom-
atous degeneration, giant cell tumor, and superimposition of
another tumorous condition, such as metastatic disease,
plasma cell myeloma, and lymphoma. Of these, sarcomatous
transformation was first recognized by Paget in some of his
original five patients. Its reported frequency has varied: In
patients with widespread skeletal involvement, sarcomatous
degeneration may occur in as many as 5–10%; with less
extensive skeletal disease, this complication may be apparent
in fewer than 1% of patients [60]. At present, it is generally
believed that approximately 1% of patients with Paget disease
develop malignant changes [2, 61, 62], although higher rates,
on the order of 5%, are still suggested [63]. Sarcomatous
degeneration usually is observed in patients who are 55–80
years old; it is slightly more common in men than in women,
associated with pain and swelling, typically unifocal, and most
commonly evident in the femur, innominate bone (Fig. 8), and
humerus. Also, sarcomatous degeneration is apparent in
areas of pagetic involvement. Although sarcomas in Paget
disease vary widely in their cellularity and cytologic details,
they appear to arise from the substratum of fibrous tissue in
the pagetic bone. Predominance of certain cells most com-
monly leads to a diagnosis of osteosarcoma (50–60%) or
fibrosarcoma (20–25%), although diagnoses such as chon-
drosarcoma (10%) and, rarely, sarcoma of myeloid and mes-
enchymal elements may be entertained. Osteolysis is the
dominant radiographic characteristic of the sarcoma.

Giant cell tumors in Paget disease are almost always con-
fined to the skull or the facial bones, with rare involvement of

other skeletal sites [64, 65]. Patients with this neoplasm are
generally elderly and have polyostotic Paget disease. Met-
astatic disease and Paget disease also may coexist [66],
although the precise relationship between the two conditions
is not clear.

Of the articular complications of Paget disease, osteoar-
thritis—especially in the hip (Fig. 9) and knee—is best known
[67–69]. The precise pathogenesis of the joint changes has
been debated, although some investigators have supported
the concept that a disturbance in endochondral ossification is
important [70]. Hypervascularity and rapid turnover in pagetic
subchondral bone may lead to accelerated endochondral
bone formation, which occurs at the expense of the articular
cartilage. Superimposed on this interference with dynamics
at the chondroosseous junction are mechanical abnormalities
in and around the articulation.

Finally, insufficiency (stress) fractures and acute complete
fractures may complicate Paget disease [71, 72]. These are
most commonly observed in the bones of the lower extremity,
particularly the femur (Fig. 10) and the tibia. As the risk of
sarcoma is substantial in cases of complete fracture in Paget
disease, biopsy is recommended whenever such a fracture
develops [2].

REFERENCES

1. Schmorl G. Ueber Ostitis deformans Paget. *Virchows Arch [A]*
   1932;283:694–751
2. Hamdy RC. *Paget's disease of bone: assessment and management*. New
   York: Praeger, 1981
3. Paget J. On a form of chronic inflammation of bones (osteitis deformans).
   Med Chir Tr 1877;60:37
4. Barry HC. *Paget's disease of bone*. Edinburgh: E&S Livingstone, 1969:16
5. Ziegler R, Holz G, Rotzler B, Minne H. Paget's disease of bone in West
   Germany: Prevalence and distribution. *Clin Orthop* 1985;194:199–204
6. Montagu MFA. Paget's disease (osteitis deformans) and heredity. *Am J
   Hum Genet* 1949;1:94–112
7. Groh JA. Mono-ostotic Paget's disease as a clinical entity. Roentgenologic
   observations in nine cases. *Am J Roentgenol* 1943;50:230–243 (reprinted
   AJR 1988;150:235–248)
8. Paget S. *Memoirs and letters of Sir James Paget*, 5th impression. London:
   Longmans, Green, 1901
9. Resnick D, Niwayama G. Paget's disease. In: Resnick D, Niwayama G,
   eds. *Diagnosis of bone and joint disorders*. Philadelphia: Saunders,
   1981:1721–1754
10. Mirra JM. Pathogenesis of Paget's disease based on viral etiology. *Clin
    Orthop* 1987;217:162–170
11. Rebel A, Malkani K, Basle M, Bregeon C. Osteoclast ultrastructure in
    Paget's disease. *Calcif Tissue Res* 1976;20:187–199
12. Mills BG, Singer FR. Nuclear inclusions in Paget's disease of bone. *Science*
    1976;194:201–202
13. Mills BG, Singer FR, Weiner LP, Suffin S, Stabile E, Holst P. Evidence for
    both respiratory syncytial virus and measles virus antigens in the osteo-
    clasts of patients with Paget's disease of bone. *Clin Orthop* 1984;183:
    303–311
14. Rebel A, Basle M, Pouplard A, Kouyoumdjian S, Filmon R, Lepatezour A.
    Viral antigens in osteoclasts from Paget's disease of bone. *Lancet*
    1980;2:344–346
15. Mirra JM, Bauer H, Grant TT. Giant cell tumor with viral-like intranuclear
    inclusions associated with Paget's disease. *Clin Orthop* 1981;158:
    243–251
16. Mirra JM, Gold RH. Case Report 186. *Skeletal Radiol* 1982;8:67–70
17. Schajowicz F, Ubios AM, Araujo ES, Cabrini RL. Virus-like intranuclear
    inclusions in giant cell tumor of bone. *Clin Orthop* 1985;201:247–250
18. Fornasier VL, Flores L, Hastings D, Sharp T. Virus-like filamentous intra-
    nuclear inclusions in giant cell tumor, not associated with Paget's disease
    of bone. A case report. *J Bone Joint Surg [Am]* 1985;67-A:333–336

19. Rame CS, Powers TM, Kinuko S. Acute multiple sclerosis: Confirmation of "paramyxovirus like" intranuclear inclusions. Arch Neurol 1974;30: 39–46

20. Rebel A, Basle M, Poupiard A, Malkani K, Filmon R, Lepatezour A. Toward a viral etiology for Paget's disease of bone. Metab Bone Dis Relat Res 1981;3:235–238

21. Singer FR, Mills BG. Evidence for a viral etiology of Paget's disease of bone. Clin Orthop 1983;178:245–251

22. Lentle BC, Russell AS, Hesip PG, Percy JS. The scintigraphic findings in Paget's disease of bone. Clin Radiol 1966;27:129–135

23. Khairi MRA, Wellman HN, Robb JA, Johnston CC. Paget's disease of bone (ostellis deformans). Symptomatic lesions and bone scan. Ann Intern Med 1973;79:348–351

24. Miller SW, Castronovo FP, Pendergrass IIP, Potsaid MB. Technetium-99m labeled diphosphonate bone scanning in Paget's disease. Am J Roentgenol Radium Ther Nucl Med 1974;121:177–183

25. Fletcher JW, Butler RL, Henry RE, Solario-George E. Donati RM. Bone marrow scanning in Paget's disease. J Nucl Med 1973;14:928–930

26. Andrews JT. Osteoporosis circumscripta crani: its importance in brain scintiscanning. Australas Radiol 1976;20:273–280

27. Vellenga CJLR, Pauwels EKJ, Bijvoet OLM. Some characteristics of local scintigraphic and radiologic patterns of Paget's disease of bone (osteitis deformans). Diagn Imag Clin Med 1985;54:273–281

28. Dionne D, Tallefer R, Lebland R, Chartrand R. Osteoporosis circumscripta crani: a cause for "doughnut" sign on cerebral and bone scans. Clin Nucl Med 1983;8:377

29. Lander PH, Hadjipavlou AG. Paget's disease with contraction of long bones. Radiology 1986;159:471–472

30. Schubert F, Siddle KJ, Harper JS. Diaphyseal Paget's disease: an unusual finding in the tibia. Clin Radiol 1984;35:71–74

31. Schlesinger A, Naimark A, Lee VW. Diaphyseal presentation of Paget's disease in long bones. Radiology 1983;147:83–84

32. Moser RP, Vinh TN, Ros PR, Smirniotopoulos JA, Madewell JE, Barrey BH. Paget's disease of the anterior tibial tubercle. Radiology 1987;164:211–214

33. Maldague B, Malghem J. Dynamic radiologic patterns of Paget's disease of bone. Clin Orthop 1987;217:126–151

34. Lander PH, Hadjipavlou AG. A dynamic classification of Paget's disease. J Bone Joint Surg [Br] 1986;68-B:431–438

35. Zadek RE, Migram JW. Progression of Paget's disease in the tibia. A case report. J Bone Joint Surg [Am] 1976;58-A:876–878

36. Maldague B, Malghem J. Radiological aspects of Paget's disease of bone. Acta Orthop Belg 1974;40:425–453

37. Doyle FH, Banks LM, Pennock JM. Radiologic observations on bone resorption in Paget's disease. Arthritis Rheum 1980;23:1205–1214

38. Hamilton CR Jr. Effects of synthetic salmon calcitonin in patients with Paget's disease in bone. Am J Med 1974;56:315–322

39. Mallette LE. An unusual morphologic evolution of Paget's disease of bone. Arthritis Rheum 1981;24:1544

40. White MP, Daniels EH, Murphy WA. Osteolytic Paget's bone disease in a young man. Rapid healing with human calcitonin therapy. Am J Med 1985;78:326–332

41. Johnston CC Jr, Khairi MRA, Meunier RJ. Use of etidronate (EHDP) in Paget's disease of bone. Arthritis Rheum 1980;23:1172–1176

42. Siris ES, Canfield RE, Jacobs TP, Baquiran DC. Long term therapy of Paget's disease of bone with EHDP. Arthritis Rheum 1980;23:1177–1184

43. Russell RG, Smith R, Preston C, Walton RJ, Woods CG. Diphosphonates in Paget's disease. Lancet 1974;1:894–898

44. Hosking D, Bijvoet OL. Combined diphosphonate and calcitonin therapy for Paget's disease of bone. Calcif Tissue Res 1976;21[Suppl]:321–326

45. Smith R, Russell RG, Bishop M. Diphosphonates and Paget's disease of

46. Canfield R, Rosner W, Skinner J, et al. Diphosphonate therapy of Paget's disease of bone. Clin Endocrinol Metab 1977;44:96–106

47. Johnston CC Jr, Altman RD, Canfield RE, Finerman GAM, Tauber JD, Elbert ML. Review of fracture experience during treatment of Paget's disease of bone with etidronate disodium (EHDP). Clin Orthop 1981;172:186–194

48. Boyce BF, Smith L, Fogelman I, Johnston E, Ralston S, Boyle IT. Focal osteomalacia due to low-dose diphosphonate therapy in Paget's disease. Lancet 1984;1:821–824

49. Douglas DL, Duckworth T, Kanis JA, et al. Biochemical and clinical responses to dichloromethylene diphosphonate (Cl₂MDP) in Paget's disease of bone. Arthritis Rheum 1980;23:1185–1192

50. Ryan WG, Schwartz. Mithramycin treatment of Paget's disease of bone: exploration of combined mithramycin-EHDP therapy. Arthritis Rheum 1980;23:1155–1161

51. Frame B, Marel GM. Paget's disease: a review of current knowledge. Radiology 1981;141:21–24

52. Vallenga CJLR, Pauwels EKJ, Bijvoet OLM, Hosking DJ, Frigink WB. Bone scintigraphy in Paget's disease treated with combined calcitonin and diphosphonate (EHDP). Metab Bone Dis Relat Res 1982;4:103–112

53. Lee JY. Bone scintigraphy in evaluation of Didronel therapy in Paget's disease. Clin Nucl Med 1981;6:356–358

54. Altman RD. Musculoskeletal complications of Paget's disease of bone. Arthritis Rheum 1980;23:1121–1127

55. Weiss GM. Lumbar spinal canal stenosis in Paget's disease. Spine 1983;8:192–198

56. Smidek HH. Neurologic and neurosurgical sequelae of Paget's disease of bone. Clin Orthop 1977;127:70–77

57. Zlatkin MB, Lander PH, Hadjipavlou AG, Levine JS. Paget's disease of the spine: CT with clinical correlation. Radiology 1986;160:155–159

58. Clarke PRR, Williams HI. Ossification in extradural fat in Paget's disease of the spine. Br J Surg 1975;62:571–572

60. Price CHG, Goldie W. Paget's sarcoma of bone. J Bone Joint Surg [Br] 1969;51-B:205–224

61. Wick MP, McLeod RA, Siegal GP, Greditzar HG III, Unni KK. Sarcomas of bone complicating osteitis deformans (Paget's disease). Fifty years experience. Am J Surg Pathol 1981;5:47–59

62. Greditzar HG III, McLeod RA, Unni KK, Beabout JW. Bone sarcomas in Paget's disease. Radiology 1983;146:327–333

63. Schajowicz F, Santini Araujo E, Berenstein M. Sarcomas complicating Paget's disease of bone. A clinicopathological study of 62 cases. J Bone Joint Surg [Br] 1983;65-B:299–307

64. Bonakdarpour A, Harwick R, Pickering J. Case Report 34. Skeletal Radiol 1977;2:52–55

65. Francis R, Lewis E. CT demonstration of giant cell tumor complicating Paget's disease. J Comput Assist Tomogr 1983;7:917–918

66. Roberts JA. Paget's disease and metastatic carcinoma. A case report. J Bone Joint Surg [Br] 1986;68-B:22–23

67. Machtey I, Rodman GP, Benedek TG. Paget's disease of the hip joint. Am J Med Sci 1966;251:524–531

68. Graham J, Harris WH. Paget's disease involving the hip joint. J Bone Joint Surg [Br] 1971;53-B:650–659

69. Roper BA. Paget's disease involving the hip joint. A classification. Clin Orthop 1971;80:33–38

70. Goldman AB, Bullough P, Kammermans S, Ambos M. Osteitis deformans of the hip joint. AJR 1977;128:601–606

71. Barry HC. Orthopedic aspect of Paget's disease of bone. Arthritis Rheum 1981;23:1128–1130

72. Stevens J. Orthopedic aspects of Paget's disease. Metab Bone Dis Relat Res 1981;3:271–278

Principles of Bone Biology

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CHAPTER 69

Paget's Disease of Bone

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Clinical Aspects

The Patient

Paget's disease is a common disorder of the skeleton that is focal in nature and extremely variable in its clinical manifestations (Singer and Krane, 1990). The majority of patients are without symptoms. When symptoms are present, skeletal deformity and pain are most common. Deformities are usually most apparent in the skull, face, and lower extremities. Pain is of several origins. Generalized bone pain is surprisingly uncommon, but joint pain (hips and knees) is seen not infrequently due to degenerative arthritis. Pain of neural or spinal origin is unusual but the most severe.

A variety of complications may first bring the patient to medical attention. The complications depend both on the affected skeletal sites and the overall extent of the disease (three or fewer bones are commonly involved). Patients with Paget's disease in the skull often develop a hearing deficit if the temporal bone is affected. Massive enlargement of the cranium is associated with basilar impression and neurological impairment. Vertebral involvement may produce compression fractures, spinal stenosis, neurological impairment, and degenerative arthritis. Paget's disease in the pelvis and femurs commonly is associated with degenerative arthritis of the hips. Involvement of the femur and tibia may lead to pathological fractures of these long bones. Nonunion of a femoral fracture is relatively common. Degenerative arthritis in the knees is also a common feature when lower extremity long bones are extensively involved by Paget's disease. The most serious complication is the development of a sarcoma which fortunately only occurs in <1% of patients. It always arises in a pagetic lesion and not in unaffected bone.

Systemic complications of Paget's disease generally occur with more extensive disease. Hypercalcemia, preceded by hypercalciuria, usually is noted with total bed rest and is related to the accelerated bone resorption induced by immobilization. Increased cardiac output and, less commonly congestive heart failure are a consequence of the great vascularity of bones affected by Paget's disease. Hyperuricemia has been observed in males with extensive disease and may reflect increased purine turnover.

Recent evidence suggests that Paget's disease occurs much more commonly in families than was previously reported. An extensive investigation of the relatives of 35 patients with Paget's disease in Madrid revealed that 40% of the patients had at least one first-degree relative with the disease (Morales-Piga et al., 1995). All studies of familial Paget's disease suggest an autosomal dominant inheritance. No genetic abnormality has been defined as yet that explains familial Paget's disease, but genetic marker studies in a family in Northern Ireland suggest linkage of the disease to the long arm of chromosome *18*

(Hughes *et al.*, 1994). This family probably has a variant of Paget's disease that results in a more aggressive clinical course with an earlier age of onset (Osterberg *et al.*, 1988).

Radiology and Nuclear Medicine

The diagnosis of Paget's disease is primarily accomplished by roentgenographic evaluation of the skeleton. Over the years it has become clear that the disease evolves through several stages as observed by serial roentgenograms.

The initial stage of the disease is represented by a localized area of reduced bone density often referred to as an osteolytic lesion. This is most readily detected in the skull where it is found as a discrete round or oval lesion in the frontal or occipital bones. It is called *osteoporosis circumscripta*. Paget's disease in the long bones almost always begins in the subchondral region of either epiphysis (uncommonly both may be affected simultaneously). The osteolytic process has then been seen to advance proximally or distally at ~1 cm/year in the untreated patient. The advancing front usually has a V-shaped or arrowhead appearance.

In the most advanced stage of Paget's disease, the areas of previous osteolytic dominance now are characterized by a chaotic sclerotic appearance, a phase that is called osteoblastic or osteosclerotic. In long bones the osteolytic phase is commonly seen preceding the osteosclerotic region when much of the bone has been affected by the disease. Another feature of this phase is considerable thickening of the sclerotic bone, which can reach monumental proportions in the skull. Osteolytic activity of a secondary nature often can be observed as clefts in the thickened bone. It is likely that the evolution of the disease into its most severe form occurs over much of the life span of the patient. Two other features of the disease have been noted through serial roentgenographic observations. Although the disease can slowly course through an entire bone, it does not cross a joint space to affect an adjacent bone. Also, it is extremely rare for new lesions of Paget's disease to be detected at any site in the skeleton after the diagnosis and extent of the disease has been determined initially.

Bone scans are the most sensitive means of detecting pagetic lesions. Radiolabeled bisphosphonates accumulate in regions where blood flow and bone formation are increased and can outline early lesions that are not detectable on roentgenograms. Radioactive gallium can also define areas of Paget's disease activity because of uptake of gallium by osteoclasts.

Histopathology

Beginning with the observations of Schmorl in 1932, it has become appreciated that the osteoclast is the dominant cell in the pathogenesis of Paget's disease. Osteo-

clasts are increased in number in the Haversian canals of the cortex in the absence of other abnormalities and are in great number at the leading edge of the osteolytic front. The osteoclasts in Paget's disease may be far greater in size than osteoclasts in normal bone and contain as many as 100 nuclei in a cross-section as compared with two or three nuclei in a normal osteoclast. Osteoclasts of Paget's disease have a characteristic ultrastructural abnormality (Gherardi *et al.*, 1980; Howatson and Fornasier, 1982; Mills and Singer, 1976; Rebel *et al.*, 1974). This consists of microfilaments, sometimes grouped in a paracrystalline array, located in the nucleus and sometimes in the cytoplasm of osteoclasts (Figure 1). These microfilaments are not seen in non-pagetic bone or bone marrow cells. These inclusions closely resemble nucleocapsids of viruses of the paramyxoviridae family, a group of RNA viruses responsible for some of the most common childhood diseases. Despite the finding of these inclusions in the vast majority of patients studied, the budding off of an infectious virus from the osteoclasts has not been reported.

Osteoblasts are another prominent feature of the cellular pathology of Paget's disease. Large numbers of osteoblasts are often found near areas of resorbed bone and may even be prominent in a lesion that appears purely osteolytic by X-ray. The osteoblasts are usually prism-shaped or polyhedral and contain abundant rough endoplasmic reticulum, mitochondria, and a well-developed Golgi zone. These signs of cellular activity are consistent with the increased bone formation in active lesions established by the use of double labeling with tetracycline.

In addition to the increased numbers of osteoclasts and osteoblasts, the marrow of pagetic lesions tends to be grossly abnormal. The normal hematopoietic elements are usually absent and replaced by mononuclear cells of indeterminate origin intermixed with highly vascular connective tissue.

The bone matrix in Paget's disease is highly abnormal in structure and arises as a consequence of disordered bone resorption and formation. The matrix consists of a "mosaic" of irregularly shaped pieces of lamellar bone with an erratic pattern of cement lines. The matrix is interspersed with numerous foci of woven bone, which, in adults, is ordinarily found associated with fracture healing.

Biochemistry

The biochemical findings in Paget's disease help to provide an integrated assessment of the cellular events occurring thoughout the skeleton of affected patients.

Historically, the earliest index of bone matrix resorption was measurement of urinary hydroxyproline excretion while ingesting a low gelatin diet. This index is well correlated with the extent of the disease despite the fact that hydroxyproline is a prominent component of extraskeletal connective tissue as well as skeletal collagen. Measurement of collagen crosslink degradation products
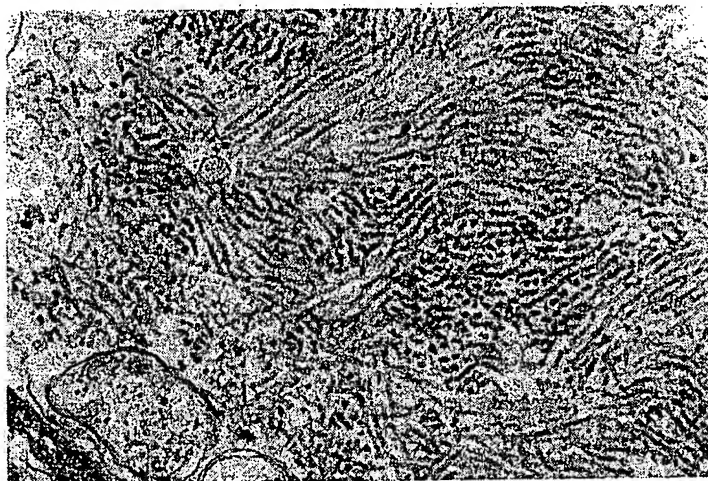
Figure 1 Electron micrograph of a nuclear inclusion in an osteoclast of a patient with Paget's disease. The microfilaments are seen both in longitudinal array and in paracrystalline array in cross section. [Provided by Dr. Barbara G. Mills.]

in urine provides more specific measurements of skeletal matrix. Urinary N-telopeptide, pyridinoline, and deoxypyridinoline have all been reported to be more specific indices of skeletal matrix resorption and are not influenced by dietary gelatin. (Alvarez et al., 1995; Garnero et al., 1994)

Serum tartrate-resistant acid phosphatase, presumably released by osteoclasts, appears to be another index of bone resorption in Paget's disease but is not routinely available, (Kraenzlin et al., 1990).

Osteoblast activity can be assessed by measurement in the serum of total alkaline phosphatase activity, bone-specific alkaline phosphatase activity, osteocalcin concentration and type 1 carboxy-terminal procollagen peptide concentration. The most useful of these serum markers are the total alkaline phosphatase and bone-specific alkaline phosphatase (Alvarez et al., 1995; Garnero and Delmas, 1993) For reasons that are not understood, serum osteocalcin is a poor index of disease activity; similarly, the type I carboxy-terminal procollagen assay is insensitive to increases in bone turnover and does not change reproducibly as the disease is suppressed by medical therapy.

Therapy

Salmon calcitonin by injection and etidronate disodium by the oral route are both effective medications for Paget's disease. They have been in use for ~20 years. They generally suppress biochemical parameters of the disease by 50%. Intravenous pamidronate disodium and oral alendronate sodium are more recent, more potent bisphospho-

nates that can reduce bone turnover to normal in the majority of patients with Paget's disease (Singer and Minoofar, 1995).

Surgery is sometimes used in Paget's disease in patients with associated degenerative arthritis of the hip (total hip replacement) and of the knee (high tibial osteotomy). Orthopaedic and/or neurosurgical procedures may occasionally be necessary after fractures and when skull or vertebral complications are present.

Evidence for the Presence of Paramyxoviruses in Paget's Disease

In early studies, Mills and colleagues attempted to rescue an infectious virus from cells cultured from surgical specimens of Paget's disease (Mills and Singer, unpublished observations; Mills et al., 1979). After trypsinization or after growing cells from explants of pagetic bones, cells were cultivated for months and then cocultivated with cell lines used to isolate Paramyxoviridae viruses. These studies failed to demonstrate an infectious virus in pagetic bone. Perhaps this is not surprising since no anatomic evidence of an infectious virus has been found in any specimen of Paget's disease and the mononuclear cells cultured from surgical specimens rarely were found to exhibit nuclear inclusions. Mills and colleagues (1985) also attempted to develop an animal model of Paget's disease by injecting crushed bone extracts or lysates from cells cultured from pagetic bone into the tibiae of athymic

nude mice. Nothing resembling Paget's disease was induced in the mouse bone, although one cell line did produce an osteosarcoma-like lesion reproducibly.

Despite the failure to find an infectious (mature) virus in specimens of Paget's disease, abundant evidence has been generated using immunohistological and molecular biological techniques that indicates the presence of viral antigens and mRNA in Paget's disease. Initially Rebel and colleagues (1980a) used a variety of antisera directed against measles virus to demonstrate measles antigen in the osteoclasts of 20 patients with Paget's disease, but not in one patient with psoriasis and another with a healing fracture. Positive results were obtained with both indirect immunofluorescence (Figures 2A and 2B) and immunoperoxidase techniques (Rebel et al., 1980b). Mills and colleagues (1980, 1981) initially reported that cell cultures from pagetic bone as well as bone sections from 12 patients were positive when stained with an antiserum against respiratory syncytial virus but not with antisera against measles virus, parainfluenza viruses, influenza A and B, rubella, mumps, and herpes simplex. Subsequently Mills and colleagues (1984) did observe that measles virus nucleocapsid protein antigens were present in the osteoclasts and/or cultured bone cells of most patients with Paget's disease. Of particular interest was their finding that in serial sections of pagetic bone, both measles virus and respiratory syncytial virus nucleocapsid antigens were demonstrable in the same osteoclasts. This observation could not be explained by cross-reactivity of the measles virus monoclonal antibodies used in this study with respiratory syncytial virus antigens and vice versa. In other immunohistological studies, Basle and colleagues (1985) demonstrated not only measles virus antigens in

6/6 specimens of Paget's disease but also simian virus 5 in 6/6 and parainfluenza 3 in 3/6. No reactivity with respiratory syncytial virus, mumps virus, influenza virus, or adenovirus type 5 antibodies was found in this study. Subsequently Basle did find a positive response with a monoclonal antibody against respiratory syncytial virus provided by Mills (personal communication). He also noted the presence of measles virus and respiratory syncytial virus antigens in the same osteoclasts on serial sections of pagetic bone. Recently Mills and colleagues (1994) have studied immunohistological staining of long-term cultures of marrow from patients with Paget's disease and from normal subjects with respect to viral antigens. In 12/12 patients measles virus and/or respiratory syncytial virus nucleocapsid antigens were found in 40–50% of the mononuclear cells and/or multinucleated cells in culture. In the control subjects, <5% of cells were positive. In a preliminary report, Reddy and colleagues (1995a) found measles virus transcripts in peripheral blood mononuclear cells in 4/5 patients and 0/10 normal subjects.

In the past 10 years, the techniques of molecular biology have increasingly been applied to the issue of the identity of the osteoclast inclusions of Paget's disease. In the first study, Basle and colleagues (1986) used in situ hybridization with a cloned measles virus cDNA probe specific for the nucleocapsid protein to search for measles virus RNA sequences in the bone of five patients. These sequences were detected in 80–90% of the osteoclasts in these specimens. Surprisingly 30–40% of the mononuclear cells in these bone specimens also had detectable mRNA sequences of measles virus nucleocapsid protein. Evidence of the measles virus was found in osteoblasts,

Figure 2 (A) A section of decalcified pagetic bone incubated with rabbit anti-LBC serum followed by goat antiserum coupled to fluorescein isothiocyanate. The LBC strain of measles virus was isolated from a patient with SSPE. The osteoclast shows a strong positive punctate (arrows) fluorescent reaction. (B) A section of decalcified pagetic bone was treated as in A except nonimmune rabbit serum was used instead of anti-LBC serum. There is no fluorescent reaction. [Reproduced with permission from Rebel et al., 1980b.]

osteocytes, fibroblasts, lymphocytes, and monocytes. Negative results were obtained in three subjects who had fluorosis, fracture healing, and hyperparathyroidism, respectively.

Because an epidemiological study in England indicated that patients with Paget's disease were more likely to have had a pet dog in the past (O'Driscoll and Anderson, 1985), Gordon and colleagues (1991) used in situ hybridization to examine the possibility that canine distemper virus might be involved in Paget's disease. Canine distemper virus is a paramyxovirus with considerable structural homology to measles virus. Bone biopsies from 27 patients and 6 patients with other bone disorders (primary hyperparathyroidism, prostatic carcinoma, osteoporosis, osteomalacia and fracture healing) were studied. Sense and antisense RNA probes to the nucleocapsid and fusion genes of the canine distemper virus were used as well as RNA probes to mRNA sequences of measles virus nucleocapsid protein. There was no demonstrable cross-reactiivity of these probes with the heterologous virus under the experimental conditions used by the investigators. Eleven of 25 patients showed hybridization with the antisense but not the sense canine distemper virus fusion protein probe. Ten of 26 patients also showed hybridization with the antisense but not the sense probe for canine distemper virus nucleocapsid protein. The canine distemper virus probes produced hybridization in ~80% of multinucleated osteoclasts, 60% of osteoblasts, and in osteocytes as well as marrow mononuclear cells (monocytes, lymphocytes). No significant hybridization was found with the measles virus probe, and none of the control specimens reacted with any of the above probes. In a subsequent study, Gordon and colleagues (1992) obtained pagetic bone for RNA extraction, reverse transcribed the RNA, and specifically amplified for canine distemper virus and measles virus sequences using the polymerase chain reaction (PCR) technique. They found that 8/13 patients had canine distemper virus nucleic acid sequences and 1/10 patients had measles virus nucleic acid sequences in the bone specimens. One patient had both. Dideoxy sequencing of the canine distemper virus PCR products revealed 2% base pair changes in a 187-bp fragment from within position 1231-1464 of the nucleocapsid gene as compared with the Onderstpoort strain of canine distemper virus. Further support of the hypothesis that canine distemper virus could be involved in the pathogenesis of Paget's disease was sought by examining the bones of dogs with distemper infections (Mee et al., 1992). In 2/4 dogs studied, in situ hybridization of metaphyseal specimens revealed strong signals with sense and antisense probes for the nucleocapsid and phosphoprotein genes. The osteoclasts were strongly positive but osteoblasts, osteocytes and bone marrow cells were also positive. Mee and colleagues (1993) also have found canine distemper virus transcripts in the bone cells of dogs with metaphyseal osteopathy. This is an acute disorder affecting young rapidly growing dogs whose signs include fe-

ver, anorexia, and painful swollen metaphyses. The histology does not resemble Paget's disease. Although the presence of canine distemper virus transcripts in dogs with distemper or metaphyseal osteopathy does not relate directly to Paget's disease, it does indicate that a paramyxovirus can infect mammalian bone cells. This has also been demonstrated in vitro by incubating canine distemper virus with canine bone marrow (Mee et al., 1995).

Additional evidence of paramyxovirus nucleocapsid transcripts in Paget's disease has come from the recent studies of Reddy and colleagues. They studied bone marrow mononuclear cells obtained from aspirations of the iliac crests of 6 patients with radiologically demonstrable Paget's disease and from the aspirations of 10 normal subjects (Reddy et al., 1995b). Using the reverse transcriptase-PCR techniques they observed that 5/6 patients had measles virus nucleocapsid transcripts whereas none of the 10 normal subjects had detectable transcripts. Dideoxy sequencing of the PCR fragments revealed several mutations within the position 1360-1371 bp of the nucleocapsid gene as compared with the Edmonston strain of measles virus. These mutations are in the same region as the mutations reported in the canine distemper virus nucleocapsid gene. Since granulocyte macrophage colony forming units (CFU-GM), the most likely osteoclast precursors, circulate in the peripheral blood Reddy and colleagues (1995a) examined peripheral blood samples for the presence of measles virus nucleocapsid transcripts by reverse transcriptase-PCR in Paget's disease and control subjects. In 4/5 patients, measles virus transcripts were detected. They were localized to peripheral blood monocytes (whose precursor is CFU-GM) by in situ hybridization. Studies were negative in 10 control subjects.

Inexplicably 2 studies from the United Kingdom and United States study have produced negative results with respect to detection of Paramyxoviridae mRNA in Paget's disease. In one study, RNA extracts of 10 bone specimens failed to exhibit measles virus, canine distemper virus, respiratory syncytial virus, or parainfluenza 3 virus transcripts after reverse transcriptase-PCR evaluation (Ralston et al., 1991). In a second study, both bone cells cultured from pagetic explants and bone biopsies were studied similarly by reverse transcriptase-PCR techniques for the presence of measles virus and canine distemper virus transcripts (Birch et al., 1994). Completely negative results were obtained. In the third study Nuovo and colleagues (1992) also could not detect measles virus-specific cDNA in pagetic specimens using PCR and in situ hybridization in combination. The explanation for the disparate results is not apparent.

Several studies have addressed the levels of circulating antibodies against various paramyxoviruses in patients with Paget's disease. Antibody titers have not been found to be greater in Paget's disease than in control subjects. (Basle et al., 1983; Gordon et al., 1993; Pringle et al., 1985).

Cellular and Molecular Biology of Paget's Disease

The development of *in vitro* techniques for the study of the ontogeny of human osteoclasts has made it possible to gain new insights into the pathogenesis of Paget's disease. Kukita and colleagues (1990) first established long-term cultures of marrow from involved bones from patients with Paget's disease and noted that the multinucleated cells that formed shared many of the characteristics of pagetic osteoclasts. As compared with osteoclast-like cells formed in normal marrow cultures, the pagetic osteoclast-like cells formed more rapidly and in much greater numbers (10- to 100-fold greater), had increased numbers of nuclei and had higher levels of tartrate-resistant acid phosphatase. Examination of these cells by electron microscopy did reveal many features of osteoclasts found in pagetic bone biopsies, but the characteristic nuclear and cytoplasmic inclusions were not observed. As previously mentioned, the antigens of measles virus and respiratory syncytial virus nucleocapsids were detectable in these cells (Mills *et al.*, 1994); apparently the nucleocapsid structures do not form in this *in vitro* setting.

Because the increased numbers of osteoclasts in pagetic lesions are of obvious importance in the pathogenesis of the disease, it seemed logical to examine osteoclast precursors in the marrow aspirates to determine if they were abnormal or whether other cells in the marrow microenvironment were participants in the pathology. Demulder and colleagues (1993) examined CFU-GM in cultures of unfractionated marrow mononuclear cells and found that CFU-GM colony formation was significantly increased compared with that of normal cells. Using an antibody that recognizes the CD34 antigen present on most hematopoietic precursors, they also isolated enriched hematopoietic precursors and found similar numbers of osteoclast precursors in pagetic and normal marrow aspirations. Subsequent coculture experiments with highly purified hematopoietic precursors (CD34+ cells) and nonhematopoietic marrow accessory cells (CD34− cells) demonstrated the growth of pagetic precursors was significantly enhanced by both normal or pagetic CD34− cells. CFU-GM colony formation was also significantly increased when normal CE34+ cells were cocultured with pagetic, but not normal, CD34− cells. CFU-GM colony-derived cells from pagetic patients also formed osteoclast-like multinucleated cells with 1,25 dihydroxyvitamin D_3 concentrations one-tenth of that required for normal multinucleated cell formation. Thus these experiments suggest that osteoclast precursors are abnormal in Paget's disease and that other cells in the pagetic marrow microenvironment may stimulate the growth and differentiation of these abnormal precursors (Figure 3).

A strong candidate for a significant autocrine/paracrine factor involved in the increased osteoclast formation in Paget's disease is interleukin-6. Roodman and colleagues (1992) found that conditioned media from long-term pagetic marrow cultures increased multinucleated cell formation in normal marrow cultures and that antibodies to
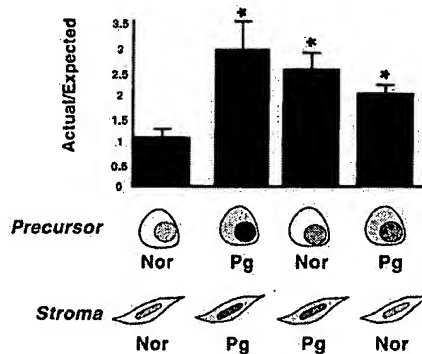

Figure 3 Summary of coculture studies using highly purified osteoclast precursors from normal and pagetic patients and stromal cells from normal and pagetic patients. Coculture of pagetic osteoclast precursors with normal or pagetic stromal cells produces a significantly increased number of CFU-GM-derived colonies over the expected number. When highly purified normal osteoclast precursors are cocultured with normal stromal cells, the expected number of CFU-GM-derived colonies are formed. In contrast, when normal osteoclast precursors are cocultured with pagetic marrow stromal cells, increased CFU-GM-derived colonies are formed. These data suggest that pagetic marrow stromal can increase the growth of both normal and pagetic osteoclast precursors and that the pagetic stromal cells, can enhance the growth of normal osteoclast precursors above expected levels. The results are presented as the ratio of actual over the expected number of CFU-GM colonies formed. The asterisk denotes a significant difference from the normal cell combination. [Adapted from Demulder *et al.*, 1993.]

interleukin-6 blocked the stimulatory activity. Antibodies to interleukin-1, GM-CSF and TNF-α had no effect on the stimulatory activity. In situ hybridization studies demonstrated that the multinucleated cells in the pagetic marrow cultures were actively transcribing interleukin-6 mRNA. In addition, bone marrow plasma samples obtained from sites of Paget's disease had increased levels of interleukin-6 in 9/10 patients as compared with samples from normal subjects. Peripheral plasma also had elevated interleukin-6 levels in 17/27 patients. In another study, basal plasma interleukin-6 activity was increased in 19/22 patients (Schweitzer et al., 1995). The concept that interleukin-6 may be an important autocrine/paracrine factor in Paget's disease is also supported by the studies of Hoyland and colleagues (1994), who used in situ hybridization to localize the expression of interleukin-6, interleukin-6 receptor, and interleukin-6 transcription factor in the bone of patients with Paget's disease in comparison with those with osteoarthritis. The osteoblasts in both disorders expressed all three mRNAs but in Paget's disease interleukin-6 and its receptor mRNA showed higher levels of expression. In the osteoclasts of both disorders, the receptor and transcription factor were expressed but only in Paget's disease was interleukin-6 mRNA expressed in osteoclasts. Hoyland and Sharpe (1994) also examined the expression of c-fos protooncogene in the bone of six patients with Paget's disease by in situ hybridisation. c-fos has been found to be important in the regulation of osteoclasts and was markedly upregulated in pagetic osteoclasts and, to a lesser extent, in the osteoblasts. It is possible that this is a consequence of interleukin-6 action (Korholz et al., 1992).

Ralston and colleagues (1994) also studied cytokine and growth factor expression in bone explants of Paget's disease and in control subjects (postmenopausal women with and without osteoporosis and young bone graft patients) and could not find differences between pagetic and nonpagetic bone. Interleukin-6 mRNA was not detected in 40% of the pagetic specimens from severely affected individuals. As in the case of the negative paramyxovirus studies reported by these investigators, there is no obvious explanation for their nonconfirmatory data.

The Etiology of Paget's Disease

As has been reviewed, Paget's disease is a focal disorder that often occurs in multiple family members. In most affected individuals, it progresses slowly over many years without extending to new sites of involvement. The underlying pathophysiology appears to reflect a localized increased in the numbers of osteoclasts followed by a secondary increase in osteoblastic activity. The osteoclasts have striking characteristics by both light and electron microscopy. In Paget's disease the osteoclasts may be far greater in size than osteoclasts in normal individuals or in patients with diseases in which osteoclasts are activated

such as primary hyperparathyroidism. A correlate of this observation is the increased number of nuclei noted in the pagetic osteoclast. The nuclei and cytoplasm harbor microfilamentous inclusions that are structurally identical to the nucleocapsids of viruses of the Paramyxviridae family. However, the structure of a mature Paramyxoviridae virus has never been reported in bone biopsy or cell culture specimens. Immunohistological and molecular techniques have indicated the presence of Paramyxoviridae proteins and mRNA in patients with Paget's disease. Finally interleukin-6 has been implicated as a potential important mediator of osteoclast function in Paget's disease. Based on this information, it seems reasonable to propose the hypothesis that Paget's disease represents a slow virus infection of bone.

Slow (or persistent) viral infections of the nervous system have been studied since the mid-1960s when Bjorn Sigurdsson, an Icelandic veterinary virologist, identified several viral disorders in sheep which exhibited a prolonged incubation period before the occurrence of symptoms. In man, the best studied disorder of this type is subacute sclerosing panencephalitis (SSPE). A small percentage of children experience an often fatal neurological syndrome ~5 years after a classical measles infection (ter Meulen et al., 1983). It proved quite difficult to rescue an infectious virus from brain specimens from patients with SSPE although nucleocapsid-like structures similar to those in Paget's disease were abundant. Persistent infection of human glioma cell lines with measles virus has been shown to result in the induction of interleukin-6 (Schneider-Schaulies et al., 1993). This observation may be relevant to Paget's disease. Examination of measles virus genes from a brain specimen of a SSPE patient revealed that nearly 2% of the nucleotides were mutated during persistence and that 35% of these mutations resulted in amino acid changes (Cattaneo et al., 1988). This also appears analogous to findings in Paget's disease.

The existence of persistent viral infections of the nervous system provides a model for pursuing a similar pathogenesis of Paget's disease. An acute viral infection with one of several paramyxoviruses could result in the establishment of a persistent viral infection in one or more bone and/or bone marrow cells, particularly in genetically susceptible individuals. This could trigger the initial osteoclastic stimulus with interleukin-6 as an important intermediary. Subsequently the normal osteoblastic response to bone resorption may become overzealous in response to the continuing pathologic osteoclastic activity.

Many issues need to be resolved before the viral hypothesis of Paget's disease can be accepted. The characteristic osteoclast nuclear and cytoplasmic inclusions of Paget's disease have also been noted in some patients with giant cell tumors of bone (Schajowicz et al., 1985), pycnodysostosis (Beneton et al., 1987), osteopetrosis (Mills et al., 1988), and primary oxalosis (Bianco et al., 1992). Does this mean that the inclusions are merely inci-

dental findings in all these disorders or in the appropriate genetic setting does a persistent viral infection trigger the pathology?

It is of considerable importance to determine the complete sequences of the Paramyxoviridae proteins and genes found in the pagetic specimens. This would clarify whether there were two viruses in many lesions, whether gene recombination occurs, or whether mutations over time account for the finding of measles and respiratory syncytial virus antigens in the same osteoclasts.

In the past there has been no evidence that canine distemper virus is responsible for any human disorder. The finding of antibodies to canine distemper virus in the circulation of patients with Paget's disease and in control subjects is consistent with this possibility. These antibody titers did not correlate with antibodies to measles virus in these individuals (Gordon et al., 1993).

Of particular interest is what accounts for the focal nature of Paget's disease when circulating mononuclear cells can be demonstrated to contain measles virus transcripts? It seems reasonable to propose that the marrow microenvironment is critical for maintenance of the localized lesions. It remains for future studies to fully define the cytokines stimulated by the bone cells and marrow stroma which interact to produce the florid advancing pathology of a pagetic lesion.

Clearly it will require many more investigations into the mechanism of the proliferation of osteoclasts in Paget's disease before the etiology of this common skeletal problem will be understood fully.

References

Alvarez, L., Guanabens, N., Peris, P., Monegal, A., Bedini, J. L., Deulofeu, R., Martinez De Osaba, M. J., Munoz- Gomez, J., Rivera- Fillat, F., and Ballesta, A. M. (1995). Discriminative value of biochemical markers of bone turnover in assessing the activity of Paget's disease. *J. Bone Miner. Res.* **10**, 458–465.

Basle, M. F., Fournier, J. G., Rozenblatt, S., Rebel, A., and Bouteille, M. (1986). Measles virus RNA detected in Paget's disease bone tissue by *in situ* hybridization. *J. Gen. Virol.* **67**, 907–913.

Basle, M. F., Rebel, A., Filmon, R., and Pilet, P. (1983). Maladie osseuse de Paget. Anticorps seriques anti-rougeole. *Presse Med.* **12**, 769–770.

Basle, M., Rebel, A., Pouplard, A., Kouyoumdjian, S., Filmon, R., and Lepatezour, A. (1979). Mise en evidence d'antigenes viraux de rougeole dans les osteoclastes de la maladie osseuse de Paget. *C. R. Acad. Sci.* **289**, 225–228.

Basle, M. F., Russell, W. C., Goswami, K. K. A., Rebel, A., Giraudon, P., Wild, F., and Filmon, R. (1985). Paramyxovirus antigens in osteoclasts from Paget's bone tissue detected by monoclonal antibodies. *J. Gen. Virol.* **66**, 2103–2110.

Beneton, M. N. C., Harris, S. C., and Kanis, J. A. (1987). Paramyxovirus-like inclusions in two cases of pycnodysostosis. *Bone* **8**, 211–217.

Bianco, P., Silvestrini, G., Ballanti, P., and Bonucci, E. (1992). Paramyxovirus-like nuclear inclusions identical to those of Paget's disease detected in giant cells of primary oxalosis. *Virchows Arch. A Pathol. Anat. Histopathol.* **421**, 427–433.

Birch, M. A., Taylor, W., Fraser, W. D., Ralston, S. H., Hart, C. A., and Gallagher, J. A. (1994). Absence of paramyxovirus RNA in

cultures of pagetic bone cells in pagetic bone. *J. Bone Miner. Res.* **9**, 11–16.

Cartwright, E. J., Gordon, M. T., Freemont, A. J., Anderson, D. C., and Sharpe, P. T. (1993). Paramyxoviruses and Paget's disease. *J. Med. Virol.* **40**, 133–141.

Cattaneo, R., Schmid, A., Eschle, D., Baczko, K., ter Meulen, V., and Billeter, M. A. (1988). Biased hypermutation and other genetic changes in defective measles viruses in human brain infections. *Cell* **56**, 255–265.

Demulder, A., Takahashi, S., Singer, F. R., Hosking, D. J., and Roodman, G. D. (1993). Abnormalities in osteoclast precursors and marrow accessory cells in Paget's disease. *Endocrinology* **133**, 1978–1982.

Garnero, P. and Delmas, P. D. (1993). Assessment of the serum levels of bone alkaline phosphatase with a new immunoradiometric assay in patients with metabolic bone disease. *J. Clin. Endocrinol. Metab.* **77**, 1046–1053.

Garnero, P., Shih, W. J., Gineyts, E., Karpf, D. B., and Delmas, P. D. (1994). Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. *J. Clin. Endocrinol. Metab.* **79**, 1693–1700.

Gherardi, G., Lo Cascio, V., and Bonucci, E. (1980). Fine structure of nuclei and cytoplasm of osteoclasts in Paget's disease of bone. *Histopathology* **4**, 63–74.

Gordon, M. T., Anderson, D. C., and Sharpe, P. T. (1991). Canine distemper virus localised in bone cells of patients with Paget's disease. *Bone* **12**, 195–201.

Gordon, M. T., Mee, A. P., Anderson, D. C., and Sharpe, P. T. (1992). Canine distemper virus transcripts sequenced from pagetic bone. *Bone Miner.* **19**, 159–174.

Gordon, M. T., Bell, S. C., Mee, A. P., Mercer, S., Carter, S. D., and Sharpe, P. T. (1993). Prevalence of canine distemper antibodies in the pagetic population. *J. Med. Virol.* **40**, 313–317.

Howatson, A. F. and Fornasier, V. L. (1982). Microfilaments associated with Paget's disease of bone: Comparison with nucleocapsids of measles virus and respiratory syncytial virus. *Intervirology* **18**, 150–159.

Hoyland, J. A., Freemont, A. J., and Sharpe, P. T. (1994). Interleukin-6, IL-6 receptor, and IL-6 nuclear factor gene expression in Paget's disease. *J. Bone Miner. Res.* **9**, 75–80.

Hoyland, J. and Sharpe, P. T. (1994). Upregulation of c-fos protooncogene expression in pagetic osteoclasts. *J. Bone Miner. Res.* **9**, 1191–1194.

Hughes, A. E., Shearman, A. M., Weber, J. L., Barr, R. J., Wallace, R. G. H., Osterberg, P. H., Nevin, N. C., and Mollan, R. A. B. (1994). Genetic linkage of familial expansile osteolysis to chromosome 18q. *Hum. Mol. Gen.* **3**, 359–361.

Korholz, D., Gerdon, S., Enczmann, J., Zessack, N., and Burdach, S. (1992). Interleukin-6 induced differentiation of a human B cell line into Ig M secreting plasma cells is mediated by cfos. *Eur. J. Immunol.* **22**, 607–610.

Kraenzlin, M. E., Lau, K.-H. W., Liang, L., Freeman, T. K., Singer, F. R., Stepan, J., and Baylink, D. J. (1990). Development of an immunoassay for human serum osteoclastic tartrate-resistant acid phosphatase. *J. Clin. Endocrinol. Metab.* **71**, 442–451.

Kukita, A., Chenu, C., McManus, L. M., Mundy, G. R., and Roodman, G. D. (1990). Atypical multinucleated cells form in long-term marrow cultures from patients with Paget's disease. *J. Clin. Invest.* **85**, 1280–1286.

Mee, A. P., Gordon, M. T., May, C., Bennett, D., Anderson, D. C., and Sharpe, P. T. (1993). Canine distemper virus transcripts detected in the bone cells of dogs with metaphyseal osteopathy. *Bone* **14**, 59–67.

Mee, A. P., Hoyland, J. A., Baird, P., Bennett, D., and Sharpe, P. T. (1995). Canine bone marrow cell cultures infected with canine distemper virus: An *in vitro* model of Paget's disease. *Bone* **17**, 461S–466S.

Mee, A. P. and Sharpe, P. T. (1994). Letter to the editor. *Bone Miner.* 24, 75–76.

Mee, A. P., Webber, D. M., May, C., Bennett, D., Sharpe, P. T., and Anderson, D. C. (1992). Detection of canine distemper virus in bone cells in the metaphyses of distemper-infected dogs. *J. Bone Miner. Res.* 7, 829–834.

Mills, B. G., Frausto, A., Singer, F. R., Ohsaki, Y., Demulder, A., and Roodman, G. D. (1994). Multinucleated cells formed in vitro from Paget's bone marrow express viral antigens. *Bone* 15, 443–448.

Mills, B. G., Holst, P. A., Stabile, E. K., Adams, J. S., Rude, R. K., Fernie, B. F., and Singer, F. R. (1985). A viral antigen-bearing cell line derived from culture of Paget's bone cells. *Bone* 6, 257–268.

Mills, B. G. and Singer, F. R. (1976). Nuclear inclusions in Paget's disease of bone. *Science* 194, 201–202.

Mills, B. G. and Singer, F. R. (1987). Critical evaluation of viral antigen data in Paget's disease of bone. *Clin. Orthop. Rel. Res.* 217, 16–25.

Mills, B. G., Singer, F. R., Weiner, L. P., and Holst, P. A. (1979). Long-term culture of cells from bone affected by Paget's disease. *Calcif. Tissue Int.* 29, 79–87.

Mills, B. G., Singer, F. R., Weiner, L. P., and Holst, P. A. (1980). Cell cultures from bone affected by Paget's disease. *Arthritis Rheum.* 23, 1115–1120.

Mills, B. G., Singer, F. R., Weiner, L. P., and Holst, P. A. (1981). Immunohistological demonstration of respiratory syncytial virus antigens in Paget disease of bone. *Proc. Natl. Acad. Sci. USA* 78, 1209–1213.

Mills, B. G., Singer, F. R., Weiner, L. P., Suffin, S. C., Stabile, E., and Holst, P. (1984). Evicence for both respiratory syncytial virus and measles virus antigens in the osteoclasts of patients with Paget's disease of bone. *Clin. Orthop. Rel. Res.* 183, 303–311.

Mills, B. G., Yabe, H., and Singer, F. R. (1988). Osteoclasts in human osteopetrosis contain viral-nucleocapsid-like nuclear inclusions. *J. Bone Miner. Res.* 3, 101–106.

Morales-Piga, A. A., Rey-Rey, J. S., Corres-Gonzalez, J., Garcia-Sagredo, J. M., and Lopez-Abente, G. (1995). Frequency and characteristics of familial aggregation of Paget's disease of bone. *J. Bone Miner. Res.* 10, 663–670.

Nuovo, M. A., Nuovo, G. J., MacConnell, P., Forde, A., and Steiner, G. C. (1992). In situ analysis of Paget's disease of bone for measles-specific PCR-amplified cDNA. *Diagh. Mol. Pathol.* 1, 256–265.

O'Driscoll, J. B. and Anderson, D. C. (1985). Past pets and Paget's disease. *Lancet* 2, 919–921.

Osterberg, P. H., Wallace, R. G. H., Adams, D. A., Crone, R. S., Dickson, G. R., Kanis, J. A., Mollan, R. A. B., Nevin, N. C., Sloan, J., and Toner, P. G. (1988). Familial expansile osteolysis—a new dysplasia. *J. Bone Joint Surg.* 70, 255–260.

Pringle, C. R., Wilkie, M. L., and Elliot, R. M. (1985). A survey of respiratory syncytial virus and parainfluenza virus type 3 neutralising and immunoprecipitating antibodies in relation to Paget disease. *J. Med. Virol.* 17, 377–386.

Ralston, S. H., Digiovine, F. S., Gallacher, S. J., Boyle, I. T., and Duff,

G. W. (1991). Failure to detect paramyxovirus sequences in Paget's disease of bone using the polymerase chain reaction. *J. Bone Miner. Res.* 6, 1243–1248.

Ralston, S. H., Hoey, S. A., Gallacher, S. J., Adamson, B. B., and Boyle, I. T. (1994). Cytokine and growth factor expression in Paget's disease: Analysis by reverse-transcription/polymerase chain reaction. *Br. J. Rheum.* 33, 620–625.

Rebel, A., Basle, M., Pouplard, A., Kouyoumdjian, S., Filmon, R., and Lepatezour, A. (1980). Viral antigens in osteoclasts from Paget's disease of bone. *Lancet* 2, 344–346.

Rebel, A., Basle, M., Pouplard, A., Malkani, K., Filmon, R., and Lepatezour, A. (1980). Bone tissue in Paget's disease of bone. Ultrastructure and immunocytology. *Arthritis Rheum.* 23, 1104–1114.

Rebel, A., Malkani, K., and Basle, M. (1974). Anomalies nucleaires des osteoclasts de la maladie osseuse de Paget. *Nouv. Presse Med.* 3, 1299–1301.

Reddy, S. V., Singer, F. R., Mallete, L., and Roodman, G. D. (1995a). Detection of measles virus transcripts in peripheral blood mononuclear cells and marrow mono-nuclear cells from patients with Paget's disease. *J. Bone Miner. Res.* 10, S154.

Reddy, S. V., Singer, F. R., and Roodman, G. D. (1995b). Bone marrow mononuclear cells from patients with Paget's disease contain measles virus nucleocapsid messenger ribonucleic acid that has mutations in a specific region of the sequence. *J. Clin. Endocrinol. Metab.* 80, 2108–2111.

Roodman, G. D., Kurihara, N., Ohsaki, Y., Kukita, A., Hosking, D., Demulder, A., Smith, J. F., and Singer, F. R. (1992). Interleukin-6, a potential autocrine/paracrine factor in Paget's disease of bone. *J. Clin. Invest.* 89, 46–52.

Schajowicz, F., Ubios, A. M., Aranjo, E. S., and Cabrini, R. L. (1985). Virus-like inclusions in giant cell tumor of bone. *Clin. Orthop. Rel. Res.* 201, 247–250.

Schmorl, G. (1932). Über Ostitis deformans Paget. *Virchows Arch. Pathol. Anat. Phsiol.* 283, 694–751.

Schneider-Schaulies, J., Schneider-Schaulies, S., and ter Meulen, V. (1993). Differential induction of cytokines by primary and persistent measles virus infections in human glial cells. *Virology* 195, 219–228.

Schweitzer, D. H., Oostendorp-Van de Ruit, M., Van Der Pluijm, G., Lowik, C. W. G. M., and Papapoulos, S. E. (1995). Interleukin-6 and the acute phase response during treatment of patients with Paget's disease with the nitrogen-containing bisphosphonate dimethylaminohydroxypropylidene bisphosphonate. *J. Bone Miner. Res.* 10, 956–962.

Singer, F. R. and Krane, S. M. (1990). Paget's disease of bone. In "Metabolic Bone Disease" (L. V. Avioli and S. M. Krane, eds.), pp. 546–615. W. B. Saunders, Philadelphia.

Singer, F. R. and Minoofar, P. (1995). Bisphosphonates in the treatment of disorders of mineral metabolism. *Adv. Endocrinol. Metab.* 6, 259–288.

ter Meulen, V., Stephenson, J. R., and Kreth, H. W. (1983). Subacute sclerosing panencephalitis. *Compr. Virol.* 18, 105–185.

CANINE DISTEMPER IN TERRESTRIAL CARNIVORES: A REVIEW

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Abstract: Canine distemper virus is a member of the genus *Morbillivirus* in the family Paramyxoviridae. Canine distemper has been recorded in domestic dogs for centuries. It is now recognized as a worldwide problem of carnivores and has the second highest fatality rate of any infectious disease, after rabies, in domestic dogs. The importance of this disease in nondomestic animals has become evident with vaccine-induced infections in a variety of species and large-scale epidemics in captive and free-ranging felids. To date, canine distemper has been reported in all families of terrestrial carnivores: Canidae, Felidae, Hyaenidae, Mustelidae, Procyonidae, Ursidae, and Viverridae. Veterinarians, including those working with nondomestic carnivores, should be familiar with the clinical signs, diagnosis, and clinical management of this disease.

Key words: Canine distemper virus, morbillivirus, nondomestic species, review, vaccine-induced disease.

INTRODUCTION

Canine distemper (CD) is the most important worldwide infectious disease of domestic dogs (*Canis familiaris*), and its fatality rate is second only to that of rabies.[122] CD is caused by canine distemper virus (CDV), first isolated by Carré in 1905.[30] Clinical distemper has been known for centuries.[76] In recent years, vaccine-induced infections have occurred in a variety of species,[26–28,63,74,88,94,121,123] as have large-scale epidemics in felids.[14,113] Canine distemper virus may have the most far reaching implications of any infectious agent for susceptible free-living and captive carnivores.[93] The discoveries of related viruses, such as phocine and delphine morbilliviruses,[103,127] and CDV's similarity to the measles virus suggest viral mutability and a zoonotic potential for CDV.

HOST RANGE

Although CD occurs worldwide in carnivores, much of its natural history is unknown. Evidence of CDV infection has been reported in all families of terrestrial carnivores: Canidae, Felidae, Hyaenidae, Mustelidae, Procyonidae, Ursidae, and Viverridae.[9,24,45,54,61,83,93,97,110] The red or lesser panda (*Ailurus fulgens*) and giant panda (*Ailuropoda melanoleuca*) have been included in the Ursidae for this review.[100,101,134]

Morbidity and mortality vary greatly in carnivores. The case fatality rate of domestic ferrets (*Mustela putorius furo*) approaches 100%,[39] whereas 50–70% of infected domestic dogs may remain

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asymptomatic carriers.[59] Fatal CDV infections have also been experimentally induced in suids and primates.[13,86,135] Natural cases of CDV-induced fatal encephalitis have been documented in a Japanese macaque (*Macaca fuscata*)[136] and collared peccaries (*Tayassu tajacu*).[11] Within the U.S., regular epidemics occur in free-ranging raccoons (*Procyon lotor*), a species that may play a role in CD epidemiology in domestic dogs in that region.[67,89,114] Conversely, in regions of the world where CDV vaccination is not widely employed (e.g., parts of Africa), domestic dogs may serve as a reservoir for free-ranging wildlife.[3,113]

ETIOLOGIC AGENT, TRANSMISSION, AND PATHOGENESIS

Canine distemper virus, a relatively large (150–250 nm) single-stranded RNA virus with a lipoprotein envelope, is a morbillivirus in the family Paramyxoviridae.[59,122] Three other well-known diseases are caused by members of the *Morbillivirus* genus: measles in primates, rinderpest in artiodactylids, and peste des petits ruminants in small ruminants. Three recently discovered viruses, phocine distemper virus in seals and cetacean morbilliviruses in porpoises and dolphins, also belong to this genus.[103,127]

The major mode of CDV transmission is through aerosolization of respiratory exudate containing virus, although other body excretions and secretions (e.g., urine) can result in infection in susceptible hosts if aerosolized. Canine distemper is highly contagious, and viral shedding may follow infection for 60–90 days.[59] Transplacental infection has been documented in domestic dogs.[77] The epidemiologic role of vertical transmission in CD and whether or not such transmission can occur in nondomestic species are unknown. Although usually short-lived in the environment, the virus can sur-

vive at lower temperatures (e.g., 48 hr at 25°C and 14 days at 5°C)[117] and may be transmitted either by direct contact or by fomites.

Natural CD pathogenesis in domestic dogs has been well characterized and may be similar in nondomestic species. A systemic infection with viremia is often present. Central nervous system (CNS) involvement is variable and dependent on the host's immune response. Within 24 hr of entering the respiratory tracts, virus spreads in macrophages via local lymphatics to tonsils and bronchial lymph nodes. Replication of the virus occurs in the tonsils and bronchial lymph nodes 2–4 days postinfection; concurrently, low numbers of CDV-infected mononuclear cells are found in other lymphoid organs. Within 4–6 days, virus proliferates widely in lymphoid organs (e.g., spleen, mesenteric lymph nodes, Kupffer's cells in the liver, and the lamina propria of the stomach and small intestine) and spreads, probably through blood, to epithelial and CNS tissues within 8–9 days of infection. The pathogenesis within 9–14 days depends on the humoral and cell-mediated host immune response. Dogs with adequate antibody titers and cell-mediated cytotoxicity will clear the virus from most tissues with no clinical signs, whereas dogs with a poor immune response experience viral spread to many tissues. Dogs with an intermediate cell-mediated response and delayed humoral response will have most virus in epithelial tissues cleared as antibody titers rise. In these latter dogs, delayed CNS signs and hyperkeratosis of the foot pads ("hard pad disease") may result when virus persists in uveal, neural, and integumental tissues.[7,59]

SUSCEPTIBILITY/CLINICAL SIGNS

Clinical signs of CD are influenced by virus strain virulence, environmental conditions, host age and immune status, and the infected species' identity. In all species, the respiratory, gastrointestinal, integumentary, and CNS systems are most commonly affected. Diphasic fever and general malaise are often associated with viremia. Infections, probably secondary to leukopenia, are common and may complicate the clinical course.

Canidae

Up to 70% of infections in domestic dogs may be subclinical.[59] Mild illness, with nonspecific listlessness, partial anorexia, fever, and upper respiratory tract infection, may occur. However, the acute generalized form has a high mortality rate in domestic dogs, with a 14–18-day incubation period and an initial transient fever and leukopenia 4–7 days postinfection. Clinical signs in acute general-

ized CD are related to the respiratory and gastrointestinal systems and include conjunctivitis, pneumonia, diarrhea (often hemorrhagic), anorexia, and severe dehydration. A neurologic manifestation of CD may occur 1–3 wk after recovery from acute generalized infection.[7,125] Additionally, neurologic distemper can occur in dogs of any age that had no or mild systemic signs and may manifest as chronic progressive neurologic dysfunction in older dogs (usually over 6 yr of age). Neurologic complications depend on viral distribution in the CNS and may include hyperesthesia, cervical rigidity, seizures, cerebellar and vestibular signs, and paraparesis or tetraparesis with sensory ataxia. Myoclonus, the involuntary twitching of muscles in a forceful simultaneous contraction (often leading to "chewing gum" fits), is also highly suggestive of CD.[23] Additional signs of CDV in the domestic dog include digital hyperkeratosis (hard pads) and optic neuritis, chorioretinitis, and uveitis.[51] In young dogs, juvenile cellulitis and metaphyseal bone lesions and irregularities to the surface of teeth due to enamel hypoplasia may be evident.[18,42,84]

Nondomestic canid species vary in CDV susceptibility, although clinical signs in acute generalized disease often resemble those described for the domestic dog. Natural and/or vaccine-induced CDV-associated disease has been documented in African wild dogs (Lycaon pictus),[1,2,4,45,87,126] Australian dingos (Canis dingo),[16] South American bush dogs (Speothos venaticus),[88] maned wolves (Chrysocyon brachyurus),[123] bat-eared foxes (Otocyon megalotis),[68,90] kit foxes (Vulpes macrotis macrotis),[16] raccoon dogs (Nyctereutes procyonoides),[16,82] coyotes (Canis latrans),[38,52,53] red foxes (Vulpes vulpes),[16,80] and gray foxes (Urocyon cinereargenteus).[16,63,67] CD-related mortalities have also been suspected in free-ranging wolf (Canis lupus) pups[71] and fennec foxes (Fennecus zerda).[93] All canids may be susceptible to CDV.

Felidae

Subclinical CDV infection in domestic cats (Felis catus) has been demonstrated experimentally,[13] and CD disease has been reported sporadically in nondomestic felids.[22,35,48,58,108,124] During a 1992 CD epidemic among 74 large captive felids, infection was histopathologically confirmed in African lion (Panthera leo), tiger (Panthera tigris), leopard (Panthera pardus), and jaguar (Panthera onca).[14] Forty-seven percent of these cats became ill and 23% died, with signs of gastrointestinal, respiratory, and CNS disease. Sixty percent of ill cats manifested CNS disease with or without preceding gastrointestinal and respiratory disease. Generalized

seizure activity, the most common neurologic abnormality, usually culminated in acute death. With rare exceptions, animals that experienced mild disease and recovered had high CDV neutralizing antibody titers, whereas those that died or were euthanatized had low or no detectable titers. Hyperkeratosis of the foot pads did not appear in any case. Histopathologic lesions identified in the lungs and the CNS differed from those in canid species (see pathology section). Only members of the genus *Panthera* died, however, several mountain lions (*Felis concolor*) demonstrated vague gastrointestinal or respiratory system signs. One clinically healthy mountain lion was shown by a CDV neutralization test to have seroconverted during the epidemic. Disease was not identified in such small felid species as bobcat (*Felis rufus*), serval (*Felis serval*), and margay (*Felis wiedii*) in this collection. A serologic survey of several other private and zoologic collections revealed CDV neutralizing antibody in a variety of healthy cats, some with a past history of gastrointestinal or respiratory system disease.[14]

In 1994, a multispecies CD epidemic of unknown origin in the Serengeti ecosystem affected 30% of a population of 3,000 African lions.[65,96,113] Many of the affected lions were emaciated, but the most frequent clinical manifestations were neurologic, including grand mal seizures and myoclonus.[113] Up to 50% of lions with clinical signs may have died. Domestic dogs (up to 30,000, many of which were unvaccinated) could have transmitted the virus to spotted hyenas (*Crocuta crocuta*), which in turn may have transmitted the disease to lions.[29,61,113] Serengeti lions are nomadic and could distribute the virus over a large range.

Hyaenidae

Fatal CD has been documented in captive hyenas and in free-ranging Serengeti hyenas.[3,21,61,97,111] However, a retrospective study involving free-ranging spotted hyenas in the Masai Mara, Kenya, showed a significant rise in CDV antibodies without clinical signs or increased mortality during a period of high domestic dog mortality associated with a CD epidemic.[3]

Mustelidae

Mustelids are among the species most susceptible to CDV disease, and the clinical presentation is similar to that seen in domestic dogs, with some exceptions. Domestic ferrets and black-footed ferrets (*Mustela nigripes*) are highly susceptible to natural CDV infection and have a fatality rate close to 100%.[10,19,39] Fatal vaccine-induced disease has

also been documented in both species.[28,55] In addition to ocular and nasal discharge, diarrhea, anorexia, seizures, and myoclonus, black-footed ferrets often have severe hyperkeratosis of the foot pads, whole body erythema, and chin and groin rash with associated pruritus.[28,133] All mustelids are probably susceptible to clinical CD. There are reports of CD in American badgers (*Taxidea taxus*),[15,56] striped skunk (*Mephitis mephitits*),[41] European mink (*Mustela lutreola*) and American mink (*Mustela vison*),[94,107,121] Eurasian badgers (*Meles meles*),[16] and European otters (*Lutra lutra*).[54,116]

Procyonidae

Natural CDV infections in raccoons[37,67,75,89,112,114] and vaccine-induced infections in kinkajous (*Potos flavus*)[74] have been documented. All procyonids are probably susceptible to CDV infection, with clinical presentations resembling those in domestic dogs.[47,91,105] Cystitis with pyuria is common,[91,105] and jaundice is sometimes associated with CDV infection in raccoons.[75] Canine distemper virus is endemic in some North American raccoon populations,[37,47,67,73,89,114] so this species may be a reservoir for nondomestic zoo animals and domestic dogs. Additionally, CD must be differentiated from rabies in individual raccoons with neurologic signs.

Ursidae

Many ursids are susceptible to CDV infection on the basis of serologic surveys,[34,43,49,83,85] and clinical CD has most commonly been documented in red pandas and giant pandas,[26,27,46,69,110] although one report of clinical disease in polar bears (*Ursus maritimus*) and *Tremarctos ornatus* neonates has been published.[115] In a serologic study of polar bears in Alaska and Russia, 35.6% of 191 samples collected from 186 bears were positive for morbillivirus antibodies on the basis of the CDV microtiter neutralization test.[49] In a separate serologic study conducted in Alaska, 14% of 480 of grizzly bears (*Ursus arctos horribilis*) were seropositive, whereas none of the 40 black bears (*Ursus americanus*) tested had antibody titers.[34] Serum neutralization tests with the Onderstepoort CDV strain resulted in seroprevalences of 16% (2/12) and 36% (4/11) in captive and free-ranging Marsican brown bears (*Ursus arctos marsicanus*), respectively, in Italy.[85] A seroprevalence study of free-ranging Florida black bears (*Ursus americanus floridanus*) found 8% of 66 bears seropositive for CDV antibodies.[43]

Clinical signs of CD, whether from natural exposure or vaccine induction, may be similar in red pandas and domestic dogs,[26,27,69,118] but some differences have been described.[46] Signs include purulent

oculonasal discharge, anorexia, diarrhea, ascending paresis, and, in some cases, terminal seizures and coma.

Canine distemper virus can be fatal to captive giant pandas[110] and can also affect captive red pandas.[110] In China, CDV antibody titers were detected in one of five captive and one of three recently rescued giant pandas.[83]

Viverridae

Two viverrid species may develop CD, the binturong (*Arctictis binturong*)[31,60,64] and the masked palm civet (*Paguma larvata*).[81] There are recent anecdotal reports of vaccine-induced CD in captive binturong (R. J. Montali, unpubl. data). In the free-ranging masked palm civet, clinical signs included dehydration, dyspnea, serous oculonasal discharge, diarrhea, local alopecia, and convulsions.[81]

DIAGNOSTIC PROCEDURES

Antemortem

In domestic dogs, acute generalized CD infection is often diagnosed by clinical signs in animals not previously vaccinated. In nondomestic species, CD may be suspected on the basis of clinical signs but must be differentiated from such other diseases with respiratory, neurologic, and/or gastrointestinal manifestations as rabies, feline panleukopenia, toxoplasmosis, canine parvovirus, lead poisoning, and bacterial enteritides. Digital, nasal, and eyelid hyperkeratoses, common in infected ferrets and mink,[39,107] are highly suggestive of infection. In raccoons, foxes, and ferrets, jaundice associated with CDV infection is occasional and unique.[24,75] Absolute lymphopenia, thrombocytopenia, regenerative anemia, decreased albumin, and increased α- and γ-globulin concentrations may be present.[59,122] Low numbers of CDV inclusions may be detected in the cytoplasm (and occasionally nuclei) of stained peripheral blood cells, especially lymphocytes. Inclusion bodies may also be detected in smears prepared from conjunctival scrapings. However, inclusion bodies are unlikely to be present in either the blood or conjunctival scrapings outside of the acute phase of infection. Interstitial or alveolar lung patterns on thoracic radiographs also support a diagnosis. Central spinal fluid (CSF) may show increased protein (>25 mg/dl) and cell count (>10 cells/μl with a predominance of lymphocytes) and increased pressure associated with inflammation. Increased anti-CDV antibody in the CSF is definitive evidence of neurologic CDV infection[71] unless the blood–brain barrier has been disrupted because CDV-specific IgG is not present in the CSF of vaccinated dogs.

Serologic tests are often unrewarding in clinical CD because most nondomestic animals die before antibody titers are measurable. However, paired sera (10–14 days apart) can be tested by viral neutralization[12] or the indirect fluorescent antibody test for a four-fold rise in antibody titer.[6] Enzyme-linked immunosorbent assays have been developed to detect serum IgG and IgM antibodies to CDV[99,109,129,130] and CDV antigen.[20,109] Detection of IgM indicates recent CDV infection unless the animal was vaccinated within 3 wk of the test. The detection of IgG is more ambiguous and can indicate either vaccination or infection.

Immunohistocytochemistry is also useful in diagnosing CD.[17,78] Immunofluorescence is usually performed on cytologic smears prepared from conjunctival, tonsilar, genital, or respiratory epithelium. Other tissues in which virus may be detected antemortem with immunocytology are blood and buffy coat smears, CSF, skin, and foot pads. Viral persistence at these sites ranges from a few days postinfection in buffy coat smears to greater than 60 days in skin and foot pads.[7,59]

It is difficult to isolate CDV by routine cell culture. Virus isolation is most successful by direct cultivation of target tissues of lymphocytes and macrophages from the infected host.[10] In cultures with no cytopathic effects after 48–72 hr, fluorescent antibody tests can detect CDV.[7,10] Polymerase chain reaction should be considered for the antemortem detection and differentiation of CD.[62]

Postmortem

Lesions of CDV infection are similar in nondomestic carnivores and in domestic dogs.[24,44,107] The most significant gross lesions are pneumonia, depletion of lymphopoietic organs, and hyperkeratosis of the nose, foot pads, and eyelids. In uncomplicated CDV infection, the only consistent pathologic finding is thymic atrophy. Common histologic findings are hyperkeratosis of the nose, foot pads, and eyelids; eosinophilic inclusion bodies in many organs (most commonly cytoplasmic but occasionally intranuclear in the CNS, urinary bladder, and bronchial epithelium); lymphoid depletion; diffuse interstitial pneumonia; and perivascular lymphoplasmacytic infiltration in areas of demyelination and neuronal degeneration of the CNS. Syncytial giant cells in the lungs and CNS white matter, anterior uvea, and lymph nodes may also be present.

In contrast to histopathologic lesions identified in the domestic dog, lungs of large felids may show diffuse alveolar type 2 cell hyperplasia with intra-

cytoplasmic and intranuclear viral inclusion bodies.[14] These cells were strongly positive for CDV antigens by immunohistocytochemical staining. This cellular response appears to be unique to large felids.[14] Additionally, feline brain histopathology may lack the typical canid pattern of demyelination with astrocytosis and vascular cuffing. Most cats have had mild, patchy CNS lesions compared with those of canids.

The lung, liver, lymph nodes, brain, and spleen of any dead animal with suspected CDV infection should be collected for viral isolation and/or PCR. Immunohistocytochemistry on formalin-fixed tissues provides definitive evidence of CDV infection.[17,78] Vaccine virus can be differentiated from street virus by differential cell culture on the basis of different target cell susceptibility.[10]

CLINICAL MANAGEMENT

Canine distemper is best prevented by vaccination.[32] Currently, the vaccines commercially available in North America contain modified live CDV that is tissue culture adapted, primate tissue Vero cells adapted, or egg adapted or a canarypox-vectored CDV, in combination with modified live virus (MLV) canine adenovirus type 2, canine coronavirus, canine parainfluenza virus, and canine parvovirus.[32,95,104] MLV vaccines derived from egg-adapted and primate tissue strains of virus have generally been safer than canine tissue culture–adapted strains for nondomestic species; most vaccine-induced CDV infections result from the latter. The Fromm-D® vaccine (Solvay, Mendota Heights, Minnesota 55120, USA), containing an egg-adapted strain and taken off the market in 1994, was labeled for use in ferrets and safe in many nondomestic species.[66,131] The USDA-approved Fervac-D® (United Vaccines, Inc., Madison, Wisconsin 53744, USA), an egg-adapted strain containing vaccine for use in ferrets, has induced disease in red pandas[94] and anaphylaxis in some mustelids (notably ferrets) and viverrids (R. J. Montali, unpubl. data). It should not be used in these species nor, perhaps, in other exotic carnivores. Galaxy-D® (Solvay, currently being manufactured by Fort Dodge, Overland Park, Kansas 50501, USA for Schering-Plough, Union, New Jersey 07083, USA), a MLV tissue culture–adapted strain with simian cell substrate, has shown promise in nondomestic species and may be safe for use in some canids, procyonids, and wolf species.[105] Studies by the ad hoc American Association of Zoo Veterinarian's CDV subcommittee with Galaxy-D® showed it to be relatively safe and efficacious in maned wolves and hybrid black-footed ferret × Siberian polecat.[95]

The use of multivalent vaccines containing CDV, such as Galaxy-6-MPH-L (Solvay), should be discouraged, at least in some nondomestic species, because of possible immunosuppression and clinical disease brought about by other MLV components.[94,121]

An experimental killed vaccine, prepared with B-propriolactone–inactivated Onderstepoort strain, has good safety but variable efficacy when used in both domestic and nondomestic species,[95] but unfortunately it is available only in limited amounts to selected institutions holding such species as SSP-managed red pandas and black-footed ferrets. A subunit vaccine with CDV immune-stimulating complexes protected domestic dogs experimentally infected with CDV[40] and harbor seals (*Phoca vitulina*) exposed to phocid distemper virus-1[128] and may be useful for nondomestic species. Recombinant vaccines with vaccina,[72,120] canarypox,[104,120,132] and fowlpox[72] vectors with either CDV[104,120,132] or rinderpest virus[72] antigens have been experimentally tested in ferrets and dogs. The recombinant canarypox-vectored CDV vaccine (Merial, Ltd., Inc., Athens, Georgia 30601, USA) has been safe and efficacious and lacked interference from other canine vaccine components in initial trials and may soon be available.[104,132] However, it is presently only licensed and available as a polyvalent product[104] and cannot be recommended at this time for use in nondomestic species. A monovalent form of this canarypox-vectored CDV vaccine is being sought.[95]

Vaccination schedules for nondomestic species are based on recommendations for the domestic dog.[6,32] Domestic dogs that received colostrum as neonates should be vaccinated every 3–4 wk between 6 and 16 wk of age. Colostrum-deprived neonates should be given two vaccinations administered on a 3–4 wk interval and starting at 2 wk of age because maternal antibodies acquired in utero should be absent by 4–6 wk of age.[6]

Data on maternal antibody interference with vaccination of raccoons and ferrets suggest that a final CDV vaccine should be administered at 18–20 wk of age in raccoons and after 10 wk of age in ferrets.[57,105] This illustrates the importance of tailoring vaccination programs to a particular species' needs. Additionally, vaccination schedules may require modification during CD epidemics or periods of increased risk of exposure.

Adults should be vaccinated twice, 3–4 wk apart. Modified live CDV vaccines induce long-lived immunity in domestic dogs[50] and hybrid ferrets.[131] Consequently, the risk of adverse events, including anaphylaxis, has raised questions about the need for annual vaccination of domestic animals.[50] Yearly

vaccine boosters may be advisable in nondomestic species for which data on antibody persistence postvaccination are lacking. Assessment of the immune response (e.g., IgG antibody levels) provides a way to assess the need for booster vaccination.[130]

Vaccination of nondomestic felids is not recommended by the Felid Taxon Advisory Group, although favorable results with a monovalent recombinant form of canarypox-vectored CDV vaccine (R. J. Montali, unpubl. data) may change this. Vaccination of free-ranging potential reservoir animals (e.g., domestic dogs in Africa and raccoons in urban North American zoos) for CDV and efforts to minimize their contact with captive and wild felids may decrease the risk for CDV infection in nondomestic felids.

All CDV vaccine use for nondomestic species is extra-label except for Fervac-D® in ferrets and Distemink® (United Vaccines, Inc., Madison, Wisconsin 53744, USA) in mink. Veterinarians should consider obtaining signed consent forms prior to vaccinating pet ferrets and pretreating with diphenhydramine to lessen the severity of anaphylactic reactions (R. A. Yates, unpubl. data). Currently, a monovalent canarypox-vectored CDV recombinant vaccine holds the most promise for general exotic carnivore protection against CDV if it becomes licensed for ferrets and widely available.

Canine distemper virus is extremely susceptible to ultraviolet light, heat, desiccation, and common disinfectants (e.g., formaldehyde, ether, chloroform, phenolic compounds, and quarternary ammonium compounds). It is short lived in the environment but can survive at low temperatures (e.g., 48 hr at 25°C and 14 days at 5°C) for extended periods.[117] Good hygienic practices and the separation of potential virus-shedding animals from susceptible hosts should be instituted.

There is no specific therapy for animals with clinical CD. Nonspecific treatment is supportive and includes fluids, antibiotics (for secondary bacterial infections), and drugs to minimize CNS inflammation and seizure activity. The prognosis in acute generalized CD is often poor but depends on the virulence of the virus strain along with the identity, age, and immune status of the individual affected. Neurologic manifestations worsen the prognosis.

ZOONOTIC POTENTIAL/FUTURE DIRECTIONS

There is no definitive evidence of naturally acquired human CDV disease although asymptomatic experimental infection may occur.[98] However, there may be a relationship between subacute sclerosing panencephalitis (SSPE), multiple sclerosis (MS),

Paget's disease, and CDV infection. Dog ownership and clinical MS are statistically correlated,[36] as are dog ownership and Paget's disease.[102] However, there is no evidence of a causal relationship between CDV and MS.[25] More evidence supports an association between measles with SSPE, although such an association is still theoretical.[33,106] Nevertheless, with the new and emerging morbilliviruses, both CDV and viruses closely related to CDV in nonhuman primates,[136] tayassuids,[11] marine mammals,[127] and nondomestic felids,[14,113] there is concern about the mutability and changing epidemiology of CD. We must be cautious when working with animals potentially infected with morbilliviruses. Potential new vaccines, including DNA (plasmid) products against these agents,[119] may hold great promise as safe and effective vaccines for exotic carnivores.

LITERATURE CITED

1. Alexander, K. A., and M. J. G. Appel. 1994. African wild dogs (*Lycaon pictus*) endangered by a canine distemper epizootic among domestic dogs near the Masai Mara National Reserve, Kenya. J. Wildl. Dis. 30: 481–485.

2. Alexander, K. A., P. A. Conrad, L. A. Gardner, C. Parish, M. Appel, M. G. Levy, N. Lerche, and P. Kat. 1993. Serologic survey for selected microbial pathogens in African wild dogs (*Lycaon pictus*) and sympatric domestic dogs (*Canis familiaris*) in Masai Mara, Kenya. J. Zoo Wildl. Med. 24: 140–144.

3. Alexander, K. A., P. W. Kat, L. G. Frank, K. E. Holekamp, L. Smale, C. House, and M. J. G. Appel. 1995. Evidence of canine distemper virus infection among free-ranging spotted hyena (*Crocuta crocuta*) in the Masai Mara, Kenya. J. Zoo Wildl. Med. 26: 201–206.

4. Alexander, K. A., P. W. Kat, L. A. Munson, A. Kalake, and M. J. G. Appel. 1996. Canine distemper–related mortality among wild dogs (*Lycaon pictus*) in Chobe National Park, Botswana. J. Zoo Wildl. Med. 27: 426–427.

5. Appel, M. J. G. 1969. Pathogenesis of canine distemper. Am. J. Vet. Res. 30: 1167–1182.

6. Appel, M. J. G. 1977. Canine distemper. *In:* Kirk, R. W. (ed.). Current Veterinary Therapy, vol. 6, Small Animal Practice. W. B. Saunders, Philadelphia, Pennsylvania. Pp. 1308–1313.

7. Appel, M. J. G. 1987. Canine distemper virus. *In:* Appel, M. J. G. (ed.). Virus Infections of Carnivores. Elsevier Science Publishers B. V., New York, New York. Pp. 133–159.

8. Appel, M. J. G., E. P. J. Gibbs, S. J. Martin, V. Ter Meulen, B. K. Rima, J. R. Stephenson, and W. P. Taylor. 1981. Morbillivirus diseases of animals and man. *In:* Kustak, E., and C. Kurstak (eds.). Comparative Diagnosis of Viral Diseases, vol. 4, Vertebrate Animal and Related Vi-

ruses, Part B—RNA Viruses. Academic Press, New York, New York. Pp. 235–297.

9. Appel, M. J. G., and R. J. Montali. 1994. Canine distemper and emerging morbillivirus diseases in exotic species. Proc. Am. Assoc. Zoo Vet. 1994: 336–339.

10. Appel, M. J. G., S. Pearce-Kelling, and B. A. Summers. 1992. Dog lymphocyte cultures facilitate the isolation and growth of virulent canine distemper virus. J. Vet. Diagn. Invest. 4: 258–263.

11. Appel, M. J. G., C. Reggiardo, B. A. Summers, S. Pearce-Kelling, C. J. Mare, T. H. Noon, R. E. Reed, J. N. Shively, and C. Orvell. 1991. Canine distemper virus infection and encephalitis in javelinas (collared peccaries). Arch. Virol. 119: 147–152.

12. Appel, M. J. G., and D. S. Robson. 1973. A microneutralization test for canine distemper virus. Am. J. Vet. Res. 34: 1459–1463.

13. Appel, M. J. G., B. E. Sheffy, D. H. Percy, and J. M. Gaskin. 1974. Canine distemper virus in domesticated cats and pigs. Am. J. Vet. Res. 35: 803–806.

14. Appel, M. J. G., R. A. Yates, G. L. Foley, J. J. Bernstein, S. Santinelli, L. H. Spelman, L. D. Miller, L. H. Arp, M. Anderson, M. Barr, S. Pearce-Kelling, and B. A. Summers. 1994. Canine distemper epizootic in lions, tigers, and leopards in North America. J. Vet. Diagn. Invest. 6: 277–288.

15. Armstrong, W. H. 1942. Canine distemper in the American badger. Cornell Vet. 32: 447.

16. Armstrong, W. H., and C. H. Anthony. 1942. An epizootic of canine distemper in a zoological park. Cornell Vet. 32: 286–288.

17. Axthelm, M. K., and S. Krakowka. 1986. Immunocytochemical methods for demonstrating canine distemper virus antigen in aldehyde-fixed paraffin-embedded tissue. J. Virol. Monogr. 13: 215–229.

18. Baumgartner, W., R. W. Boyce, S. E. Weisbrode, S. Aildinger, M. K. Axthelm, and S. Krakowka. 1995. Histologic and immunocytochemical characterization of canine distemper–associated metaphyseal bone lesions in young dogs following experimental infection. Vet. Pathol. 32: 702–709.

19. Bernard, S. L., J. R. Gorham, and L. M. Ryland. 1984. Biology and diseases of ferrets. In: Fox, J. G., B. J. Cohen, and F. M. Loew (eds.). Laboratory Animal Medicine. Academic Press, New York, New York. Pp. 385–397.

20. Bernard, S. L., D. T. Shen, and J. R. Gorham. 1982. Antigen requirements and specificity of enzyme-linked immunosorbent assay for detection of canine IgG against canine distemper viral antigen. Am. J. Vet. Res. 43: 2266–2269.

21. Blair, W. R. 1907. Report of the veterinarian. N. Y. Zool. Soc. Annu. Rep. 12: 119.

22. Blythe, L. L., J. A. Schmitz, M. Roelke, and S. Skinner. 1983. Chronic encephalomyelitis caused by canine distemper virus in a Bengal tiger. J. Am. Vet. Med. Assoc. 183: 1159–1162.

23. Breazile, J. E., B. S. Blaugh, and N. Nail. 1966. Experimental study of canine distemper myoclonus. Am. J. Vet. Res. 27: 1375–1379.

24. Budd, J. 1981. Distemper. In: Davis, J. W., L. H. Karstad, and D. O. Trainer (eds.). Infectious Diseases of Wild Mammals. Iowa State University Press, Ames, Iowa. Pp. 31–44.

25. Burridge, M. J. 1978. Multiple sclerosis, house pets, and canine distemper: critical review of recent reports. J. Am. Vet. Med. Assoc. 173: 1439–1444.

26. Bush, M., R. J. Montali, O. Brownstein, A. E. James, and M. J. G. Appel. 1976. Vaccine-induced canine distemper in a lesser panda. J. Am. Vet. Med. Assoc. 169: 959–960.

27. Bush, M., and M. Roberts. 1977. Distemper in captive red pandas. Int. Zoo Yearb. 17: 194–196.

28. Carpenter, J. W., M. J. G. Appel, R. C. Erickson, and M. N. Novilla. 1976. Fatal vaccine-induced canine distemper virus infection in black-footed ferrets. J. Am. Vet. Med. Assoc. 169: 961–964.

29. Carpenter, M. A., M. J. G. Appel, M. E. Roelke-Parker, L. Munson, H. Hofer, M. East, and S. J. O'Brien. 1998. Genetic characterization of canine distemper virus in Serengeti carnivores. Vet. Immunol. Immunopathol. 65: 259–266.

30. Carré, H. 1905. Sur la maladie des jeunes chiens. C. R. Acad. Sci. 140: 689–690, 1489–1491.

31. Chandra, A. M. S., P. E. Ginn, S. P. Terrell, B. Ferguson, A. Adjirir-Awere, P. Dennis, and B. L. Homer. 2000. Canine distemper virus infection in binturongs (Arctictis binturong). J. Vet. Diagn. Invest. 12: 88–91.

32. Chappuis, G. 1995. Control of canine distemper. Vet. Microbiol. 44: 351–358.

33. Chen, T. T., I. Watanabe, W. Zeman, and J. Mealey. 1969. Subacute sclerosing panencephalitis: propagation of measles virus from brain biopsy in tissue culture. Science 163: 1193–1194.

34. Chomel, B. B., R. W. Kasten, G. Chappuis, M. Soulier, and Y. Kikuchi. 1998. Serological survey of selected canine viral pathogens and zoonoses in grizzly bears (Ursus arctos horribilis) and black bears (Ursus americanus) from Alaska. Rev. Sci. Tech. Off. Int. Epizoot. 17: 756–766.

35. Cook, R. D., and G. E. Wilcox. 1981. A paramyxovirus-like agent associated with demyelinating lesions in the CNS of cats. J. Neuropathol. Exp. Neurol. 40: 328.

36. Cook, S. D., and P. C. Dowling. 1977. A possible association between house pets and multiple sclerosis. Lancet 1: 980–982.

37. Cranfield, M. R., I. K. Barker, K. G. Mehren, and W. A. Rapley. 1984. Canine distemper in wild raccoons (Procyon lotor) at the Metropolitan Toronto Zoo. Can. Vet. J. 25: 63–66.

38. Cypher, B. L., J. H. Scrivner, K. L. Hammer, and T. P. O'Farrell. 1998. Viral antibodies in coyotes from California. J. Wildl. Dis. 34: 259–264.

39. Davidson, M. 1986. Canine distemper, virus infection in the domestic ferret. Compend. Contin. Educ. Pract. Vet. 8: 448–453.

40. de Vries, P., F. G. C. M. Uytdehaag, and A. D. M. E. Osterhaus. 1988. Canine distemper virus (CDV) immune-stimulating complexes (iscoms), but not measles vi-

rus iscoms, protect dogs against CDV infection. J. Gen. Virol. 69: 2071–2083.

41. Diters, R. W., and S. W. Nielsen. 1978. Toxoplasmosis, distemper, and herpesvirus infection in a skunk (Mephitis mephitis). J. Wildl. Dis. 14: 132–136.

42. Dubielzig, R. R., R. J. Higgins, and S. Krakowka. 1981. Lesions of the enamel organ of developing dog teeth following experimental inoculation of gnotobiotic puppies with canine distemper virus. Vet. Pathol. 18: 684–689.

43. Dunbar, M. R., M. W. Cunningham, and J. C. Roof. 1998. Seroprevalence of selected disease agents from free-ranging black bears in Florida. J. Wildl. Dis. 34: 612–619.

44. Dungworth, D. L. 1993. The Respiratory System, vol. 3, Canine Distemper Virus. In: Jubb, K. V. F., P. C. Kennedy, and N. Palmer (eds.). Pathology of Domestic Animals. Academic Press, Inc., New York, New York. Pp. 617–624.

45. Durchfeld, B., W. Baumgartner, W. Herbst, and R. Brahm. 1990. Vaccine-associated canine distemper infection in a litter of African hunting dogs (Lycaon pictus). J. Med., Series B37: 203–212.

46. Erken, A. H. M., and E. F. Jacobi. 1972. Successful breeding of lesser panda (Ailurus fulgens F. Cuvier, 1825) and loss through inoculation. Bijdr. Dierkd. 42: 92–95.

47. Evans, R. H. 1984. Studies of a virus in a biological system: naturally occurring and experimental canine distemper in the raccoon (Procyon lotor). M. S. Thesis, Southern Illinois Univ., Carbondale, Illinois.

48 Fix, A. S., D. P. Riordan, H. T. Hill, M. A. Gill, and M. B. Evans. 1989. Feline panleukopenia virus and subsequent canine distemper virus infection in two snow leopards (Panthera uncia). J. Zoo Wildl. Med. 20: 273–281.

49. Follmann, E. H., G. W. Garner, J. F. Evermann, and A. J. McKeirnan. 1996. Serological evidence of morbillivirus infection of polar bears (Ursus maritimus) from Alaska and Russia. Vet. Rec. 138: 615–618.

50. Ford, R. B., and R. D. Schultz. 2000. In: Bonagura, J. D. (ed.). Kirk's Current Veterinary Therapy, vol. 3, Small Animal Practice. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 250–253.

51. Gelatt, K. N., R. D. Whitley, D. A. Samuelson, and G. Garcia-Sanchez. 1985. Ocular manifestations of viral diseases in small animals. Compend. Contin. Educ. Pract. Vet. 7: 968–976.

52. Gese, E. M., R. D. Schultz, M. R. Johnson, E. S. Williams, R. L. Crabtree, and R. L. Ruff. 1997. Serological survey for diseases in free-ranging coyotes (Canis latrans) in Yellowstone National Park, Wyoming. J. Wildl. Dis. 33: 47–56.

53. Gese, E. M., R. D. Schultz, O. J. Rongstad, and D. E. Andersen. 1991. Prevalence of antibodies against canine parvovirus and canine distemper virus in wild coyotes in southeastern Colorado. J. Wildl. Dis. 27: 320–323.

54. Giesel, O. 1979. Distemper in otters. Berl. Muench. Tieraerztl. Wochenschr. 92: 304. (In German.)

55. Gill, J. M., W. J. Hartley, and N. L. Hodgkinson. 1988. An outbreak of post-vaccinal suspected distemper-like encephalitis in farmed ferrets (Mustela putorius furo). N. Z. Vet. J. 36: 173–176.

56. Goodrich, J. M., E. S. Williams, and S. W. Buskirk. 1994. Effects of a modified-live virus canine distemper vaccine on captive badgers (Taxidea taxus). J. Wildl. Dis. 30: 492–496.

57. Gorham, J. R. 1999. Some experiments and field observations of distemper in mink and ferrets. Adv. Vet. Med. 41: 557–570.

58. Gould, D. H., and W. R. Fenner. 1983. Paramyxovirus-like nucleocapsids associated with encephalitis in a captive Siberian tiger. J. Am. Vet. Med. Assoc. 183: 1319–1322.

59. Greene, G. E., and M. J. Appel. 1990. Canine distemper. In: Greene, C. E. (ed.). Infectious Diseases of the Dog and Cat. W. B. Saunders, Philadelphia, Pennsylvania. Pp. 226–241.

60. Gross, L. J. 1948. Species susceptibility to viruses of Carré and feline enteritis. Am. J. Vet. Res. 9: 65–68.

61. Haas, L., H. Hofer, M. East, P. Wohlsein, B. Liess, and T. Barrett. 1996. Canine distemper virus infection in Serengeti spotted hyaenas. Vet. Microbiol. 49: 147–152.

62. Haas, L., S. M. Subbarao, T. G. Harder, B. Liess, and T. Barrett. 1991. Detection of phocid distemper virus RNA in seal tissue using slot hybridization and the polymerase chain reaction: genetic evidence that the virus is distinct from canine distemper virus. J. Gen. Virol. 72: 393–398.

63. Halbrooks, R. D., L. J. Swango, P. R. Schnurrenberger, F. E. Mitchell, and E. P. Hill. 1981. Response of gray foxes to modified live-virus canine distemper vaccines. J. Am. Vet. Med. Assoc. 179: 1170–1174.

64. Hamerton, A. C. 1937. Diseases due to infection. Proc. Zool. Soc. Lond. 107: 443.

65. Harder, T. C., M. Kenter, M. J. G. Appel, M. E. Roelke-Parker, T. Barrett, and A. D. M. E. Osterhaus. 1995. Phylogenetic evidence for canine distemper virus in Serengeti's lions. Vaccine 13: 521–523.

66. Harrenstien, L. A., L. Munson, E. C. Ramsay, C. F. Lucash, S. A. Kania, and L. N. D. Potgieter. 1997. Antibody responses of red wolves to canine distemper virus and canine parvovirus vaccination. J. Wildl. Dis. 33: 600–605.

67. Hoff, G. L., and W. J. Bigler. 1974. Epizootic of canine distemper virus infection among urban raccoons and gray foxes. J. Wildl. Dis. 10: 423–428.

68. Hofmeyer, G. F. B. 1956. Two hundred and eighty four autopsies at the National Zoological Gardens, Pretoria. J. S. Afr. Vet. Med. Assoc. 27: 263–282.

69. Itakura, C., K. Nakamura, J. Nakatsuka, and M. Goto. 1979. Distemper infection in lesser panda due to administration of a canine distemper live vaccine. Jpn. J. Vet. Sci. 41: 561–566.

70. Johnson, G. C., W. R. Fenner, and S. Krakowka. 1988. Production of immunoglobulin G and increased antiviral antibody in cerebrospinal fluid of dogs with delayed-onset canine distemper viral encephalitis. J. Neuroimmunol. 17: 237–251.

71. Johnson, M. R., D. K. Boyd, and D. H. Pletscher. 1994. Serologic investigations of canine parvovirus and

canine distemper in relation to wolf (*Canis lupus*) pup mortalities. J. Wildl. Dis. 30: 270–273.

72. Jones, L., E. Tenorio, J. Gorham, and T. Yilma. 1997. Protective vaccination of ferrets against canine distemper with recombinant pox virus vaccines expressing the H or F genes of rinderpest virus. Am. J. Vet. Res. 58: 590–593.

73. Karstad, L., and J. Budd. 1964. Distemper in raccoons characterized by giant-cell pneumonitis. Can. Vet. J. 5: 326–330.

74. Kazacos, K. R., H. L. Thacker, H. L. Shivaprasad, and P. P. Burger. 1981. Vaccination-induced distemper in kinkajous. J. Am. Vet. Med. Assoc. 179: 1166–1169.

75. Kilham, L, R. T. Habermann, and G. M. Herman. 1956. Jaundice and bilirubinemia as manifestations of canine distemper in raccoons and ferrets. Am. J. Vet. Res. 17: 144–148.

76. Kirk, H. 1922. Canine Distemper: Its Complications, Sequelae and Treatment. Baillere, Tindall and Cox, London, England.

77. Krakowka, S., E. A. Hoover, A. Koestner, and K. Ketring. 1977. Experimental and naturally occurring transplacental transmission of canine distemper virus. Am. J. Vet. Res. 38: 919–922.

78. Kristensen, B., and M. Vandevelde. 1978. Immunofluorescence studies of canine distemper encephalitis on paraffin-embedded tissue. Am. J. Vet. Res. 39: 1017–1021.

79. Laperle, A. 1993. Distemper chez les ratons laveurs. Med. Vet. Que. 23: 83–85.

80. Little, S. E., W. R. Davidson, E. W. Howerth, P. M. Rakich, and V. F. Nettles. 1998. Diseases diagnosed in red foxes from the southwestern United States. J. Wildl. Dis. 34: 620–624.

81. Machida, N., N. Izumisawa, T. Nakamura, and K. Kiryu. 1992. Canine distemper virus infection in a masked palm civet (*Paguma larvata*). J. Comp. Pathol. 107: 439–443.

82. Machida, N., K. Kiryu, K. Oh-ishi, E. Kanda, N. Izumisawa, and T. Nakamura. 1993. Pathology and epidemiology of canine distemper in raccoon dogs (*Nyctereutes procyonoides*). J. Comp. Pathol. 108: 383–392.

83. Mainka, S. A., Q. Xianmeng, H. Tingmei, and M. J. Appel. 1994. Serologic survey of giant pandas (*Ailuropoda melanoleuca*), and domestic dogs and cats in the Wolong Reserve, China. J. Wildl. Dis. 30: 86–89.

84. Malik, R., M. Dowden, P. E. Davis, G. S. Allan, V. R. Barrs, P. J. Canfield, and D. N. Love. 1995. Concurrent juvenile cellulitis and metaphyseal osteopathy: an atypical canine distemper virus syndrome? Aust. Vet. Pract. 25: 62–67.

85. Marsilio, F., P. G. Tiscar, L. Gentile, H. U. Roth, G. Boscaglis, M. Tempesta, and A. Gatti. 1997. Serologic survey for selected viral pathogens in brown bears from Italy. J. Wildl. Dis. 33: 304–307.

86. Matsibara, Y., Y. Morikawa, Y. Yoshikawa, K. Hagashima, and K. Yamanouchi. 1985. Encephalitis induced in non-human primates by canine distemper virus adapted to human neural cells. Jpn. J. Exp. Med. 55: 99–108.

87. McCormick, A. E. 1983. Canine distemper in Af-

rican cape hunting dogs (*Lycaon pictus*) possibly vaccine induced. J. Zoo Anim. Med. 14: 66–71.

88. McInnes, E. F., R. E. Burroughs, and N. M. Duncan. 1992. Possible vaccine-induced canine distemper in a South American bush dog (*Speothos venaticus*). J. Wildl. Dis. 28: 614–617.

89. Mitchell, M. A., L. L. Hungerford, C. Nixon, T. Esker, J. Sullivan, R. Koerkenmeier, and J. P. Dubey. 1997. Serologic survey for selected infectious disease agents in raccoons from Illinois. J. Wildl. Dis. 35: 347–355.

90. Moehlman, P. D. 1983. Socioecology of silver-backed and golden jackals (*Canis misomelas* and *Canis aureus*). In: Eisenberg, J. F., and D. G. Kleiman, (eds.). Advances in the Study of Mammalian Behavior. Special Publication 7. American Society of Mammalogists, Lawrence, Kansas. Pp. 423–453.

91. Monson, R. A., and W. B. Stone. 1976. Canine distemper in wild carnivores in New York. N.Y. Fish Game J. 23: 149–154.

92. Montali, R. J., C. R. Bartz, J. A. Teare, J. T. Allen, M. J. G. Appel, and M. Bush. 1983. Clinical trials with canine distemper vaccines in exotic carnivores. J. Am. Vet. Med. Assoc. 183: 1163–1167.

93. Montali, R. J., G. R. Bartz, and M. Bush. 1987. Canine distemper virus. In: Appel, M. J. (ed.). Virus Infections of Carnivores. Elsevier Science Publishers, Amsterdam, The Netherlands. Pp. 347–443.

94. Montali, R. J., R. G. Cambre, M. Sutherland-Smith, and M. J. G. Appel. 1994. Vaccination against canine distemper in exotic carnivores: successes and failures. Proc. Am. Assoc. Zoo Vet. 1994: 340–344.

95. Montali, R. J., W. Heuschele, E. Williams, and W. Lance. 1998. Development, production and safety evaluation of canine distemper vaccines for use in exotic carnivores final report, September 10, 1998. American Association of Zoo Veterinarians Canine Distemper Virus Subcommittee, Media, Pennsylvania.

96. Morell, V. 1994. Serengeti's big cats going to the dogs. Science 264: 1664.

97. Munson, L., M. J. G. Appel, M. A. Carpenter, S. J. O'Brien, and M. Roelke-Parker. 1995. Canine distemper in wild felids. Proc. Am. Assoc. Zoo Vet./Wildl. Dis. Assoc./Am. Assoc. Wildl. Vet. 1995: 135–136.

98. Nicolle, C. 1931. La maladie du jeune age des chiens est transmissible expérimentalement a l'homme sous forme inapparente. Arch. Inst. Pasteur Tunis 20: 312–323.

99. Noon, K. F., M. Rogul, L. N. Binn, T. J. Keefe, R. H. Marchwicki, and M. J. Appel. 1980. Enzyme-linked immunosorbent assay for evaluation of antibody to canine distemper virus. Am. J. Vet. Res. 41: 605–609.

100. Nowak, R. M. 1991. Order Carnivora. In: R. M. Nowak (ed.). Walker's Mammals of the World, 5th ed., vol. 2. The Johns Hopkins University Press, Baltimore, Maryland. Pp. 1045–1219.

101. O'Brien, S. J., W. G. Nash, D. E. Wildt, M. E. Bush, and R. E. Benveniste. 1985. A molecular solution to the riddle of the giant panda's phylogeny. Nature 317: 140–144.

102. O'Driscoll, J. B., and D. C. Anderson. 1985. Past pets and Paget's disease. Lancet 919–921.

103. Osterhaus, A. D. M. E., R. L. de Swart, H. W. Vos, P. S. Ross, M. J. H. Kenter, and T. Barrett. 1995. Morbillivirus infections of aquatic mammals: newly identified members of the genus. Vet. Microbiol. 44: 219–227.

104. Pardo, M. C., J. E. Bauman, and M. Mackowiak. 1997. Protection of dogs against canine distemper by vaccination with a canarypox virus recombinant expressing canine distemper virus fusion and hemagglutinin glycoproteins. Am. J. Vet. Res. 58: 833–836.

105. Paré, J. A., I. K. Barker, G. J. Crawshaw, S. A. McEwen, P. S. Carman, and R. P. Johnson. 1999. Humoral response and protection from experimental challenge following vaccination of raccoon pups with a modified-live canine distemper virus vaccine. J. Wildl. Dis. 35: 430–439.

106. Payne, F. E., J. V. Baublis, and H. H. Itabashi. 1969. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. N. Engl. J. Med. 281: 585–589.

107. Pearson, R. C., and J. R. Gorham. 1987. Canine distemper virus. In: Appel, M. J. (ed.). Virus Infections of Carnivores. Elsevier Science Publishers B. V., New York, New York. Pp. 371–378.

108. Piat, B. L. 1950. Susceptibility of young lions to distemper. Bull. Serv. Elev. Indust. Anim. Afrig. Occid. Fr. 3: 39–40.

109. Potgieter, L. N. D., and P. A. Ajidagba. 1989. Quantitation of canine distemper virus and antibodies by enzyme-linked immunosorbent assays using protein A and monoclonal antibody capture. J. Vet. Diagn. Invest. 1: 110–115.

110. Qui, X., and S. Mainka. 1993. Review of mortality of the giant panda (Ailuropoda melanoleuca). J. Zoo Wildl. Med. 24: 425–429.

111. Rewell, R. E. 1947. Report of the pathologist for the year 1947. Proc. Zool. Soc. Lond. 118: 507.

112. Robinson, V. B., J. W. Newberne, and D. M. Brooks. 1957. Distemper in the American raccoon (Procyon lotor). J. Am. Vet. Med. Assoc. 131: 276–278.

113. Roelke-Parker, M. E., L. Munson, C. Packer, R. Kock, S. Gleaveland, M. Carpenter, S. J. O'Brien, A. Pospischil, R. Hofmann-Lehmann, H. Lutz, G. L. M. Mwamengele, M. N. Mgasa, G. A. Machange, B. Summers, and M. J. G. Appel. 1996. A canine distemper virus epidemic in Serengeti lions (Panthera leo). Nature 379: 441–445.

114. Roscoe, D. E. 1993. Epizootiology of canine distemper in New Jersey raccoons. J. Wildl. Dis. 29: 390–395.

115. Schonbauer, M. von, S. Kobi, and A. Schonbauer-Langle. 1984. Perinatale staupeinfektion bei drei eisbären (Ursus maritimus) und bei einem brillenbaren (Tremarctos ornatus). Verhandslungsbericht Int. Symp. Erkrankungen Zootiere 26: 131–136.

116. Scott, W. A. 1979. Use of vaccines in exotic species. Vet. Rec. 104: 199.

117. Shen, D. T., and J. R. Gorham. 1980. Survival of pathogenic virus at 5°C and 25°C. Vet. Med. Small Anim. Clin. 75: 69–70.

118. Sikarskie, J. G., and G. L. Brady. 1991. Canine distemper in a vaccinated red panda (Ailuris fulgens fulgens). Proc. Am. Assoc. Zoo Vet. 1991: 292–293.

119. Sixt, N., A. Cardoso, A. Vallier, J. Fayolle, R. Buckland, and T. F. Wild. 1998. Canine distemper virus DNA vaccination induces a humoral and cellular immunity and protects against a lethal intracerebral challenge. J. Virol. 72: 8472–8476.

120. Stephensen, C. B., J. Welter, S. R. Thaker, J. Taylor, J. Tartaglia, and E. Paoletti. 1997. Canine distemper virus (CDV) infection of ferrets as a model for testing morbillivirus vaccine strategies: NYVAC- and ALVAC-based CDV recombinants protect against symptomatic infection. J. Virol. 71: 1506–1513.

121. Sutherland-Smith, M. R., B. A. Rideout, A. B. Mikolon, M. J. G. Appel, P. J. Morris, A. L. Shima, and D. J. Janssen. 1997. Vaccine-induced canine distemper in European mink, Mustela lutreola. J. Zoo Wildl. Med. 28: 312–318.

122. Swango, L. J. 1995. Canine viral diseases. In: Ettinger, S. J., and E. C. Feldman (eds.). Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 398–409.

123. Thomas-Baker, B. 1985. Vaccination-induced distemper in maned wolves, vaccination-induced corneal opacity in a maned wolf. Proc. Am. Assoc. Zoo Vet. 1985: 53.

124. Truyen, U., N. Stockhofe-Zurwieden, O. R. Kaaden, and J. Pohlenz. 1990. A case report: encephalitis in lions. Pathological and virological findings. Dtsch. Tieraerztl. Wochenschr. 97: 89–91.

125. Vandervelde, M., and M. Cachin. 1992. The neurologic form of canine distemper. In: Kirk, R. W., and J. D. Bonagura (eds.). Current Veterinary Therapy, vol. 11, Small Animal Practice. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 1003–1007.

126. Van Heerden, J., N. Bainbridge, R. E. J. Burroughs, and N. P. J. Kriek. 1990. Distemper-like disease and encephalitozoonosis in wild dogs (Lycaon pictus). J. Wildl. Dis. 25: 70–75.

127. Visser, I. K. G., M. Van Bressem, R. L. de Swart, M. W. G. van de Bildt, H. W. Vos, R. W. J. van der Heijden, J. T. Saliki, C. Orvell, P. Kitching, T. Kuiken, T. Barrett, and A. D. M. E. Osterhaus. 1993. Characterization of morbilliviruses isolated from dolphins and porpoises in Europe. J. Gen. Virol. 74: 631–641.

128. Visser, I. K. G., E. J. Vedder, M. W. G. van de Bildt, C. Orvell, T. Barrett, and A. D. M. E. Osterhaus. 1992. Canine distemper virus ISCOMs induce protection in harbour seals (Phoca vitulina) against phocid distemper but still allow subsequent infection with phocid distemper virus-1. Vaccine 10: 435–438.

129. Von Messling, V., T. C. Harder, V. Moennig, P. Rautenberg, I. Nolte, and L. Haas. 1999. Rapid and sensitive detection of immunoglobulin M (IgM) and (IgG) antibodies against canine distemper virus by a new recom-

binant nucleocapsid protein based enzyme-linked immunosorbent assay. J. Clin. Microbiol. 37: 1049–1056.

130. Warner, T. A. N. N. Schwarz Ben Meir, Z. Babichev, and L. E. Carmichael. 1998. Assessment of immunization response to canine distemper virus vaccination in puppies using a clinic-based enzyme-linked immunosorbant assay. Vet. J. 155: 171–175.

131. Williams, E. S., S. L. Anderson, J. Cavender, C. Lynn, K. List, C. Hearn, and M. J. G. Appel. 1996. Vaccination of black-footed ferret (*Mustela nigripes*) × Siberian polecat (*M. eversmanni*) hybrids and domestic ferrets (*M. putorius furo*) against canine distemper. J. Wildl. Dis. 32: 417–423.

132. Williams, E. S., and R. J. Montali. 1998. Vaccination of black-footed ferret × Siberian polecat hybrids against canine distemper with recombinant and modified-live virus vaccines. Proc. Annu. Wildl. Dis. Assoc. Conf. 1998: 107.

133. Williams, E. S., E. T. Thorne, M. J. G. Appel, and D. W. Belitsky. 1988. Canine distemper in black-footed ferrets (*Mustela nigripes*) from Wyoming. J. Wildl. Dis. 24: 385–398.

134. Wozencraft, W. C. 1993. Order Carnivora. *In:* Wilson, D. E., and D. M. Reeder (eds.). Mammal Species of the World, 2nd ed. Smithsonian Institute Press, Washington, D.C. Pp. 279–384.

135. Yamanouchi, K., Y. Yoshikawa, T. A. Sato, S. Katow, S. Kobune, K. Kobune, N. Uchida, and A. Shishido. 1977. Encephalomyelitis induced by canine distemper virus in non-human primates. Jpn. J. Med. Sci. Biol. 30: 241–257.

136. Yoshikawa, Y., F. Ochikubo, Y. Matsubara, H. Tsuruoka, M. Ishii, K. Shirota, Y. Nomura, M. Sugiyama, and K. Yamanouchi. 1989. Natural infection with canine distemper virus in a Japanese monkey (*Macaca fuscata*). Vet. Microbiol. 20: 193–205.